Remarks

Claims 1-53 are pending in the subject application. Claims 19-53 have been withdrawn from consideration as being drawn to a non-elected invention. By this Amendment, applicants have canceled claims 7-8, 11 and 17-53 without disclaimer or prejudice to applicants' rights to pursue the subject matter of these claims in this or another application. Applicants have also amended claims 1 and 9-10 and added new claim 54. Therefore, claims 1-6, 9-10, 12-16 and 54 are pending in this application.

Support for the amendment to claim 1 may be found, *inter alia*, on page 13, lines 18-19 of the subject specification.

Support for new claim 54 may be found, *inter alia*, on page 8, lines 6-13 of the subject specification.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 1-6 and 8

The Examiner rejected claims 1-6, 8 and 11 under 35 U.S.C. § 112, first paragraph, alleging that the specification, while being enabling for a monoclonal humanized antibody directed against an epitope on glatiramer acetate, allegedly does not reasonably provide enablement for any other antibody, citing an example polyclonal humanized antibody directed against an epitope on glatiramer acetate. The Examiner alleged that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The Examiner noted that claims 1-6, 8 and 11 are broadly directed to a humanized antibody directed against an epitope on glatiramer acetate. The Examiner alleged that the described process of how to make the claimed humanized antibody refers to a monoclonal antibody only, citing pages 22-24 and especially, page 22, lines 32-33, "[t]he resulting chimeric monoclonal antibody", emphasis added. The Examiner alleged that the instant specification fails to provide any guidance or working examples on how to make any other humanized antibody except for a monoclonal antibody and there is no information known from the prior art that could lead a skilled artisan in a process of producing the claimed antibody. Therefore, the Examiner alleged that, in view of the lack of guidance on the subject, one skilled in the art would have to resort to a substantial amount of undue experimentation to be able to practice the full scope of applicant's invention, as currently claimed.

In response, without conceding the correctness of the Examiner's position, applicants have amended claim 1 to recite "a monoclonal humanized antibody directed against an epitope on glatiramer acetate," which the Examiner acknowledged to be enabled. In addition, applicants have canceled claims 8 and 11 without disclaimer or prejudice to applicants' rights to pursue the subject matter of these claims in this or another application. Accordingly, applicants respectfully request that the Examiner withdraw the rejection of claims 1-6 under 35 U.S.C. § 112, first paragraph.

Claims 10-18

The Examiner rejected claims 10-18 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one

skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner noted that claims 10-18 are directed to a pharmaceutical composition comprising an antibody directed against an epitope on glatiramer acetate in an amount effective to treat a disease associated with demyelination of central nervous system axons. The Examiner noted that the instant specification describes the results of the experiments performed on mice infected with encephalomyelitis virus and treated by intraperitoneal injection with antibodies to glatiramer acetate. However, the Examiner alleged that it is not clear and not disclosed in the instant specification what is "an amount to treat a disease associated with demyelination of central nervous system axons." The Examiner alleged that, in order to extrapolate the results of improved remyelination of axons in a rodent model of experimental viral encephalitis and create a pharmaceutical composition comprising an antibody in an amount effective to treat a disease associated with demyelination of central nervous system axons, one skilled in the art would allegedly have to perform a significant amount of undue experimentation to determine the route, duration and quantity of administration of the claimed antibody to a subject. The Examiner also alleged that the instant specification does not disclose how these parameters are to be determined, how a similar method was practiced in the art with a different agent or provide a working example, prophetic or actual, of the claimed method. The Examiner alleged that, because the claimed humanized antibody directed against an epitope on glatiramer acetate appears to be novel, one skilled in the art would allegedly not be able to rely on the knowledge found in the prior art for the treatment of a disease associated with demyelination of central nervous system by administration of an

antibody to a substance not normally present within a body. The Examiner alleged that, in the absence of guidance, a practitioner would have to resort to a substantial amount of undue experimentation involving the variation in the amount, duration and route of administration of a humanized antibody directed against an epitope on glatiramer acetate of the instant invention.

The Examiner alleged that the instant situation is analogous to that which was addressed in *In re Colianni,* 195 U.S.P.Q. 150 (CCPA 1977), which held that a "[d]isclosure that calls for application of "sufficient" ultrasonic energy to practice claimed method of fusing bones but does not disclose what "sufficient" dosage of ultrasonic energy might be or how those skilled in the art might select appropriate intensity, frequency, and duration, and contains no specific examples or embodiment by way of illustration of how claimed method is to be practiced does not meet requirements of 35 U.S.C. § 112 first paragraph."

The Examiner acknowledged that the standard of an enabling disclosure is not the ability to make and test if the invention worked but one of the ability to make and use with a reasonable expectation of success. The Examiner noted that a patent is granted for a completed invention, not the general suggestion of an idea and how that idea might be developed into the claimed invention. The Examiner noted that, in *Genentec, Inc, v. Novo Nordisk,* 42 USPQ 2d 100 (CAFC 1997), the court held that: "patent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable" and that "[t]ossing out the mere germ of an idea does not constitute enabling disclosure". The Examiner noted that the court further

stated that "when there is no disclosure of any specific starting material or of any of the conditions under which a process is to be carried out, undue experimentation is required; there is a failure to meet the enablement requirements that cannot be rectified by asserting that all the disclosure related to the process is within the skill of the art" and that "[i]t is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement."

The Examiner alleged that the instant specification is not enabling because one allegedly can not follow the guidance presented therein and practice the claimed invention without first making an inventive contribution.

In reply, without conceding the correctness of the Examiner's position, applicants have amended claim 10 by removing the phrase, "in an amount effective to treat a disease associated with demyelination of central nervous system axons." Applicants have also canceled claims 11 and 17-18 without disclaimer or prejudice to applicants' rights to pursue the subject matter of these claims in this or another application. Therefore, applicants respectfully request that the Examiner withdraw the rejection of claims 10 and 12-16 under 35 U.S.C. § 112, first paragraph.

Rejections Under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 1-18 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention.

Specifically, the Examiner alleged that claims 1, 9 and 10 are vague and indefinite for the recitation of "(Copolymer 1)." The Examiner stated that it is clear that glatiramer acetate is known under different chemical and brand names. The Examiner suggested that only one name be consistently used throughout the claims. The Examiner alleged that claims 2-8 and 11-18 are indefinite for being dependent from allegedly indefinite claims.

In response, applicants have amended claims 1, 9 and 10 by removing the recitation of "Copolymer 1." Accordingly, applicants respectfully request that the Examiner withdraw this rejection under 35 U.S.C. § 112, second paragraph.

Claim Rejections under 35 U.S.C. § 103

The Examiner rejected claim 9 under 35 U.S.C. § 103(a) as allegedly unpatentable over Teitelbaum et al., 1991 (reference of IDS of Paper No. 7, page 7). The Examiner alleged that claim 9 encompasses a F_{ab} fragment that binds to an epitope on glatiramer acetate. The Examiner alleged that Teitelbaum et al. disclose a monoclonal antibody against the synthetic copolymer 1 (glatiramer acetate), citing the abstract, for example. The Examiner acknowledged that Teitelbaum et al. do not expressly disclose a F_{ab} fragment of their antibody.

The Examiner alleged that, at the time the invention was made, it would have been *prima facie* obvious to a person of ordinary skill in the art to produce a F_{ab} fragment that binds to an epitope on glatiramer acetate using a monoclonal antibody disclosed by Teitelbaum et al. The Examiner alleged that one of ordinary skill in the art would have been motivated to do this for the reason of producing antibodies for staining purposes,

for example.

In reply, applicants have amended claim 9 to be dependent on claim 1, which is directed to a humanized monoclonal antibody. Applicants contend that Teitelbaum et al. do not teach or suggest a humanized monoclonal antibody. Hence, Teitelbaum et al. do not teach or suggest a F_{ab} fragment thereof. Accordingly, applicants respectfully request that the Examiner withdraw the rejection of claim 9 under 35 U.S.C. § 103.

SECOND SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT PURSUANT TO 37 C.F.R. §1.97(c)(2)

In accordance with their duty of disclosure under 37 C.F.R. §1.56, applicants would like to direct the Examiner's attention to the following publications which are listed again on the attached Form PTO-1449 **(Exhibit A)** and copies of References 1-8 **(Exhibits 1-8)** are enclosed.

This Second Supplemental Information Disclosure Statement is being submitted after the issuance of a first Office Action on the merits in connection with the subject application, but prior to the issuance of a final action under 37 C.F.R § 1.113, a notice of allowance under 37 C.F.R. § 1.311 or an action that otherwise closes prosecution. Pursuant to 37 C.F.R. § 1.97(c)(2), the required fee for filing an Information Disclosure Statement is $180.00 and a check in this amount is enclosed. Accordingly, this Second Supplemental Information Disclosure Statement shall be considered pursuant to 37 C.F.R. §1.97(c)(2).

1. U.S. Patent No. 3,991,210, issued November 9, 1976 (Shea) **(Exhibit 1)**;

2. U.S. Patent Publication No. US-2002-0037848-A1, published March 28, 2002 (Eisenbach-Schwartz et al.) **(Exhibit 2)**;

3. U.S. Patent Publication No. US-2003-0004099-A1, published January 2, 2003 (Eisenbach-Schwartz et al.) **(Exhibit 3)**;

4. U.S. Serial No. 09/875,429, filed June 5, 2001 (Yong et al.)**(Exhibit 4)**;

5. Harrison and Hafler, "Antigen-Specific Therapy for Autoimmune Disease", Current Opin. Immunol., 2000, 12(6): 704-711 **(Exhibit 5)**;

6. Pender et al. Int. Med. Journal, 2002, 32: 554-563 **(Exhibit 6)**;

7. Van Noort et al., International Review of Cytology, 1995, 178: 127-205 **(Exhibit 7)**; and

8. Webster's II New Riverside University Dictionary, The Riverside Publishing Company, 1984, 933 **(Exhibit 8)**.

Applicants request that the Examiner review the publications and make them of record in the subject application.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number provided below.

No fee, other than the $180.00 surcharge for the Second Supplemental Information Disclosure Statement and the $110.00 surcharge for the one-month extension of time, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is deemed necessary, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

8/13/03

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Docket No. 60724-A/JPW/GJG/CSN

Application
for
United States Letters Patent

To all whom it may concern:

Be it known that, we,

V. Wee Yong and Sophie Chabot

have invented certain new and useful improvements in
THE USE OF GLATIRAMER ACETATE (COPOLYMER 1) IN THE TREATMENT OF CENTRAL NERVOUS SYSTEM DISORDERS

of which the following is a full, clear and exact description.

The Use of Glatiramer Acetate (Copolymer 1) in the Treatment of Central Nervous System Disorders

This application claims benefit of U.S. Provisional Application No. 60/209,372, filed June 5, 2000.

Throughout this application, various references are cited, using arabic numbers within parentheses. Full citations for these references can be found at the end of the specification, immediately preceding the claims. These publications, in their entireties, are hereby incorporated by reference into the application to more fully describe the state of the art to which the invention pertains.

Field of the Invention

The present invention provides methods of treating a mammalian subject having, or genetically predisposed to, an inflammatory, non-autoimmune central nervous system (CNS) disease, or alleviating the symptoms of such a disease.

Background

The nervous system of vertebrates is divided into the central nervous system, comprised of the brain and spinal cord, and the peripheral nervous system, consisting of the outlying nerves (22). The axons of most nerve cells are covered with a myelin sheath, a stack of specialized plasma membranes. Glial cells that wrap around the axons produce the myelin sheath. In the CNS, these glial cells are called oligodendrocytes. Each region of myelin formed by an individual oligodendrocyte is separated from the next region by an unmyelinated area called the node of Ranvier (22).

The myelin sheath acts as an electric insulator of the axon and all electric activity in axons is confined to the nodes of Ranvier.

One of the more common types of CNS diseases among human adults is multiple sclerosis. This condition is a demyelinating disease. Multiple sclerosis is a chronic, frequently progressive, inflammatory CNS disease characterized pathologically by primary demyelination and axonal injury. In multiple sclerosis patients, conduction of action potentials by the demyelinated neurons is slowed (22). Even though the etiology of multiple sclerosis is unknown, several immunological features of multiple sclerosis, and its moderate association with certain major histocompatibility complex alleles, has prompted the speculation that multiple sclerosis is an immune-mediated disease (17, 33, 55). An autoimmune hypothesis is supported by the experimental autoimmune (allergic) encephalomyelitis (EAE) model, where the injection of certain myelin components into genetically susceptible animals leads to T lymphocyte-mediated CNS demyelination (58).

Some researchers view activated T lymphocytes as a trigger of multiple sclerosis. They postulate that once T lymphocytes traverse the blood brain barrier (BBB) into the CNS parenchyma, they are reactivated following antigen presentation by microglia (53). The entry of leukocytes into tissues is a multi-step process that includes adhesion onto endothelial cells and transmigration across the endothelial barrier.

Recent evidence suggests that the expression of matrix metalloproteinases (MMPs) by leukocytes is required for T lymphocytes to enter the CNS parenchyma (42, 77, 88). An MMP is a proteolytic enzyme that possesses an active site where an invariant zinc binds to cysteine residues in the propeptide region of the MMP, keeping the MMP in an inactive state (50, 89). Activating agents disrupt the cysteine-zinc interaction to expose the active site so that catalysis can occur.

The inappropriate expression of MMPs is speculated to be involved in the pathogenesis of malignant gliomas, stroke and

Alzheimer's disease. Furthermore, several lines of evidence suggest a role for MMPs in the disease process in multiple sclerosis (37, 91). In this regard, immunohistochemically identified MMPs (specifically MMP-3,-7,-9 and -12), on microglia, astrocytes and infiltrating leukocytes, have now been documented by several groups to be present in the autopsied brains of multiple sclerosis subjects (4, 18, 21, 47, 57) and in the brains of EAE animals (16, 37). Analyses of serum samples reveal levels of MMP-9 to be significantly increased in multiple sclerosis patients compared to healthy controls; within the multiple sclerosis population, serum MMP-9 levels are higher during clinical relapse relative to periods of stability (40). In addition, serum MMP-9 levels are correlated with the number of gadolinium-enhancing lesions detected by magnetic resonance imaging (MRI) (40, 84).

In correspondence with a pathogenic role of MMPs, several hydroxamate-based agents developed to inhibit the activity of MMPs (e.g. GM6001, Ro31-9790 and BB-1101) were found to alleviate the incidence and severity of EAE (32, 34, 43, 52).

A prominent method of microglia activation is believed to be a non-antigen-specific interaction between these cells and T lymphocytes, which generates cytokines within the CNS milieu (19, 20, 67). This contention is supported by several findings obtained in culture studies. For instance, human T lymphocytes and human microglia interact to generate significant amounts of TNF-α and IL-10 (14, 15). The effect of T lymphocytes on microglia is equipotent to that of lipopolysaccharide, a very effective microglia stimulator. Researchers found additional support in the observation that the interaction between T lymphocytes and microglia does not require antigen or MHC restriction. Experiments have also shown that in a facial nerve resection model in mouse, T cells infiltrated the CNS and aggregated around microglia, and that this was correspondent with an increase in IL-1β and TNF-α (61). In a graft-versus-

host disease (GVHD) model, activated microglial cell clusters were invariably intimately associated with T cell infiltrates (68).

TNF-α can influence lymphocyte trafficking across endothelium by up regulating the expression of various adhesion molecules involved in this process (76), and is implicated in the process of demyelination. Indeed, TNF-α directly induces in vitro the apoptotic death of the myelin-producing cells in the brain, the oligodendrocytes (26, 45, 71), and intravitreal injection of TNF-α causes demyelination of mouse optic nerve axons (12). In addition, TNF-α is pro-inflammatory. The level of TNF-α is found to be elevated in the serum, cerebrospinal fluid, and brain lesions of multiple sclerosis patients, and is correlated with the disease activity (13, 35, 62, 72). TNF-α is also implicated in the pathogenicity of EAE - the administration of antibodies to TNF-α or soluble TNF-α receptors prevents the transfer of EAE and abrogates autoimmune demyelination (63, 69, 70).

IL-12 is another pro-inflammatory cytokine which has a key role in switching uncommitted T lymphocytes to the pro-inflammatory Th1 subset which secretes IFN-γ and TNF-α/β (91). IL-1β also promotes an inflammatory response and has been associated with multiple sclerosis (66).

The Th2 subset produces IL-4, IL-10 and IL-13, regulates humoral immunity and decreases local inflammation. Both IL-4 and IL-10 can inhibit the differentiation of naive precursors into Th1 cells (91). IL-4 promotes the activation of B lymphocytes and macrophages and also stimulates class switching of antibodies (22).

IL-13 is a Th2 cytokine with important immunomodulating activities. The best known IL-13 mediated function is its ability to drive the differentiation of naive CD4+ T cells

towards a Th2 phenotype (75). The anti-inflammatory functions of IL-13 include the suppressive effect on the production of pro-inflammatory cytokines by activated monocytes or by alveolar macrophages (81,, the induction of 15-lipoxygenase (51) and the inhibition of prostaglandin E2 (PGE2) formation (27). Functions of IL-13 in the CNS and on glial cell functions are not well defined (87).

IL-10 is an anti-inflammatory cytokine produced by a variety of cells, including monocytes/macrophages, T lymphocytes, and mast cells. In the CNS, potential sources of IL-10 include microglia (86) and astrocytes (49). IL-10 has important anti-inflammatory properties. First, IL-10 inhibits the production of proinflammatory cytokines by many cell types, including those of the mononuclear phagocytic lineage; indeed, IL-10 was shown to inhibit the production of TNF-α and IL-12 produced by monocytes, macrophages, and microglia (3, 9, 10, 24, 38). Also, IL-10 plays a role in causing T lymphocytes to undergo anergy (inactivation or unresponsiveness) (2). Other anti-inflammatory functions of IL-10 include its inhibitory effect on the process of antigen presentation. Treatment of macrophages/microglia with IL-10 down-regulated the expression of molecules essential for presentation of antigens, such as MHC class II (24) and the costimulatory molecules B7-1 and B7-2 (36). Finally, the role of IL-10 as an anti-inflammatory molecule is supported by the phenotype of IL-10-deficient mice; these mice develop chronic colitis, which appears to be mediated by the proinflammatory Th1 cells (8, 23, 39).

The afore-mentioned cytokines are inducible, meaning that they are produced in response to certain stimuli. In contrast, IL-6, which activates B-lymphocytes (22), is constitutive.

Summary of the Invention

The subject invention provides a method of treating a mammalian subject having an inflammatory, non-autoimmune central nervous

system (CNS) disease, or alleviating the symptoms of such a disease, comprising administering glatiramer acetate (Copolymer 1) to the subject in an amount and for a duration of time effective to treat the inflammatory, non-autoimmune CNS disease.

The subject invention also includes a method of treating a mammalian subject at risk of developing an inflammatory, non-autoimmune CNS disease comprising administering glatiramer acetate (Copolymer 1) to the subject in an amount and for a duration of time effective to minimize the severity of the inflammatory, non-autoimmune CNS disease that may occur in the subject or prevent its occurrence.

Further encompassed by the subject invention is a method of inhibiting the activity of a matrix metalloproteinase comprising contacting the matrix metalloproteinase with glatiramer acetate (Copolymer 1).

In addition, the subject invention contains a method of suppressing the production of a cytokine by activated T lymphocytes comprising contacting the activated T lymphocytes with glatiramer acetate (Copolymer 1) in an amount necessary to suppress cytokine production.

The subject invention also provides the use of glatiramer acetate (Copolymer 1) in the preparation of a pharmaceutical composition for the treatment of an inflammatory, non-autoimmune central nervous system (CNS) disease, or alleviation of the symptoms of such a disease, wherein said pharmaceutical composition is administered to the subject in an amount and for a duration of time effective to treat the inflammatory, non-autoimmune CNS disease in a mammalian subject.

The subject invention further concerns the use of glatiramer acetate (Copolymer 1) in the preparation of a pharmaceutical composition for treating a mammalian subject at risk of

developing an inflammatory, non-autoimmune CNS disease, wherein said pharmaceutical composition is administered to the subject in an amount and for a duration of time effective to minimize the severity of the inflammatory, non-autoimmune CNS disease that may occur in the subject or prevent its occurrence.

In addition, the subject invention includes the use of glatiramer acetate (Copolymer 1) in the preparation of a pharmaceutical composition for inhibiting the activity of a matrix metalloproteinase.

The subject invention also provides the use of glatiramer acetate (Copolymer 1) in the preparation of a pharmaceutical composition for suppressing the production of a cytokine by activated T lymphocytes.

Brief Description of the Figures

Figure 1 shows the lack of effect of Copolymer 1 (glatiramer acetate (GA)) on MMP-9 production by T lymphocytes. AIM-V conditioned media were collected from anti-CD3 activated T lyphocytes either in the absence of any drug treatment (control) or in the presence of 25 μg/ml Copolymer 1 or 1000 IU/ml recombinant interferon β-1b. Interferon β (IFN-β) inhibited T lymphocyte production of MMP-9, as previously reported (77), but Copolymer 1 did not affect MMP-9 protein levels. Similar results were obtained from monocytes after 1 day of treatment. The conditioned media at each time point were collected from cultures with equivalent amounts of T lymphocytes.

Figure 2 demonstrates that Copolymer 1 is an inhibitor of the enzymatic activity of MMP-9. The presence of Copolymer 1 in the incubation buffers during the development of the zymograms inhibits the manifestation of MMP activity. In contrast, IFNβ-1b is not a direct MMP activity inhibitor, although it decreases the production of MMP-9 as demonstrated in Figure 1. Positive controls, TIMP-1 and phenanthroline (phe), are shown to inhibit

MMP activity in this assay. Figure 2-A shows MMP-9 (the strongest band) from Baby Hamster Kidney (BHK) cells while Figure 2-B represents MMP-9 produced by T lymphocytes.

Figure 3 reveals that Copolymer 1 does not inhibit T lymphocyte migration across a fibronectin chamber. Figure 3-A demonstrates that 25 μg/ml Copolymer 1 treatment of T lymphocytes, for 1,2 or 3 days, did not affect the subsequent migration of cells across a fibronectin barrier compared to controls. In contrast, 3 days treatment of cells with IFNβ-1b (1000 IU/ml) decreased migration by 20 - 50% as reported previously (77). In Figure 3-B, 3-day pretreatment of T lymphocytes with various concentrations of Copolymer 1 similarly did not affect transmigration. Values are mean ± SEM of 3 analyzes each, and have been expressed as % of controls of the respective dose-response experiments.

Figure 4 shows that Copolymer 1 pretreatment of activated T lymphocytes suppresses IL-10 and TNF-α production that is generated in T lymphocyte - U937 interactions. Activated T lymphocytes in isolation produce undetectable IL-10 or TNF-α; PMA/IFNγ-pretreated U937 cells have detectable IL-10 but negligible TNF-α levels. Cytokines are significantly elevated in co-cultures and this is reduced dose-dependently by Copolymer 1 pretreatment of T lymphocytes. Values are mean ± SEM of triplicate analyzes.

Figure 5 reports that Copolymer 1 treatment of activated T lymphocytes also lead to the suppression of IL-4, IL-12 and IL-13 in T lymphocyte - U937 interactions. Both T lymphocytes and PMA/IFNγ-pretreated U937 cells do not secrete detectable amounts of IL-4, IL-12 or IL-13 into their conditioned medium. With co-culture, levels of these cytokines are increased, although the levels are low in comparison to those for IL-10 or TNF-α (Fig. 4.; amount of IL-4, IL-12 and IL-13 in T lymphocyte - U937 interactions are, respectively, 13, 12 and 62 pg/ml. These are

dose-dependently reduced by Copolymer 1 pretreatment of T lymphocytes.

Figure 6 demonstrates that cytokine production in co-culture of T lymphocytes with fetal human microglia is reduced by Copolymer 1. Values are mean ± SEM of 3 or 4 analyzes and are expressed as % of the mean of control T lymphocyte - microglia co-cultures (i.e. 0 μg/ml Copolymer 1). The amount of TNF-α in control T lymphocyte - microglia co-culture was 1068 ± 68 pg/ml while that for IL-10 was 139 ± 4 pg/ml.

Figure 7 reflects the effects of Copolymer 1 on cytokine production in adult human microglia and T lymphocyte co-cultures. In isolation, neither T lymphocytes nor microglia express IL-1β, IL-10 or TNF-α. In co-culture of microglia and activated T lymphocytes, the levels of cytokines induced after 24h were 1012 ± 86 pg/ml for TNF-α, 18 ± 2 pg/ml for IL-1β and 46 ± 2 pg/ml for IL-10. IL-6 is constitutively expressed in microglia (1010 ± 215 pg/ml), but not in T lymphocytes. Copolymer 1 pretreatment of T lymphocytes reduced the level of inducible cytokines in co-culture with microglia and also the level of expression of IL-6. Values are mean + SEM of triplicate cultures.

Figure 8 shows the morphology of microglia in T lymphocyte - microglia co-cultures. Figure 8-A shows that adult human microglia are mostly bipolar in morphology in culture. Figure 8-B demonstrates that T lymphocytes are present as single cells or clumps. Figure 8-C reports that when T lymphocytes are co-cultured with microglia in the absence of Copolymer 1, bipolar microglia become rounded/ameboid in morphology (some microglia are shown by arrows,. As Figure 8-D reflects, this morphological transformation is prevented by Copolymer 1 pretreatment of T lymphocytes (some bipolar microglia are indicated by arrows). All figures portray the same magnification, 400X.

Figure 9 reports that Copolymer 1 does not affect CXCR3 and CXCR4 expression on T lymphocytes. All T lymphocytes were activated at time 0 with anti-CD3 antibody and Copolymer 1 (25 ug/ml) was added to some cultures at 3 hours. Cells were removed and stained at 1 (Figures 9-A and 9-B), 2 (Figures 9C and 9-D) and 3 days (Figures 9-E and 9-F). An isotype control antibody was used to stain control or Copolymer 1-treated T lymphocytes and the fluorescence of these did not differ. Thus, only the isotype staining for control T lymphocytes is displayed.

Figure 10 demonstrates that Copolymer 1 does not affect CXCR3 and CXCR4 expression on U937 cells. Cells were treated with Copolymer 1 (25 ug/ml) or left untreated at time 0. Cells were removed and stained at 1 (Figures 10-A and 10-B) or 3 days (Figures 10-C and 10-D). An isotype control antibody was used to stain control and Copolymer 1-treated cells and the fluorsecence of these did not differ. Thus, only the isotype staining for control U937 cells is displayed.

Detailed Description of the Invention

A non-autoimmune disease refers to a condition characterized by a lack of significant immune-mediated damage to the diseased subject.

Oftentimes, an inflammatory disease is characterized as a condition in which there is increased blood flow to the affected areas, resulting in swelling and heightened temperature, which may produce pain.

As used in this application, an inflammatory, non-autoimmune disease includes any disease which impacts the central nervous system, but does not include an autoimmune component and is associated with an inflammatory response in the subject afflicted with the disease. Inflammatory, non-autoimmune diseases include, *inter alia*, Alzheimer's disease, Parkinson's disease, HIV

encephalopathy, brain tumor, glaucoma, neuropathy, dementia, central nervous system infection, central nervous system bacterial infection, meningitis, stroke, and head trauma.

The subject invention encompasses a method of treating a mammalian subject having an inflammatory, non-autoimmune central nervous system (CNS) disease, or alleviating the symptoms of such a disease, comprising administering glatiramer acetate (GA or Copolymer 1) to the subject in an amount and for a duration of time effective to treat the inflammatory, non-autoimmune CNS disease.

In one embodiment, the mammalian subject is human.

In another embodiment, the disease is Alzheimer's Disease.

In a further embodiment, the disease is Parkinson's Disease.

In yet another embodiment, the disease is HIV encephalopathy.

In a further embodiment, the disease is a brain tumor.

In another embodiment, the disease is glaucoma.

In a further embodiment, the disease is neuropathy or dementia.

In another embodiment, the disease is a CNS infection.

In one embodiment, the CNS infection is a bacterial infection.

In another embodiment, the bacterial infection is meningitis.

In a further embodiment, the disease results from stroke.

In yet another embodiment, the disease results from head trauma.

The subject invention encompasses embodiments wherein the route

of administration is oral, intravenous, intramuscular, subcutaneous, intraperitoneal, transdermal, nasal or rectal. The preferred routes of administration are oral and subcutaneous injection.

In one embodiment, the dose of glatiramer acetate (Copolymer 1) administered ranges from about .1 mg to about 1000 mg.

In another embodiment, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 1 mg to about 100 mg.

In a further embodiment, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 5 mg to about 50 mg.

In yet another embodiment, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 10 mg to about 30 mg.

In one embodiment, the dose of glatiramer acetate (Copolymer 1) administered is about 20 mg.

In another embodiment, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.05 mg/kg of body weight to about 50 mg/kg of body weight.

In a further embodiment, the dose of glatiramer acetate (Copolymer 1) administered ranges from about .1 mg/kg of body weight to about 10 mg/kg of body weight.

In an additional embodiment, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.1 mg/kg of body weight to about 1.0 mg/kg of body weight.

In another embodiment, the dose of glatiramer acetate (Copolymer 1) administered is about 0.3 mg/kg of body weight.

In one embodiment, the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every 30 days to about once every day. In a preferred embodiment, the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every 7 days to about once every day. In a more preferred embodiment, the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every day.

In another embodiment, the glatiramer acetate (Copolymer 1) is administered as part of a therapeutic regimen during which a cytokine antagonist is also administered to the subject.

The subject invention also concerns a method of treating a mammalian subject at risk of developing an inflammatory, non-autoimmune CNS disease comprising administering glatiramer acetate (Copolymer 1) to the subject in an amount and for a duration of time effective to minimize the severity of the inflammatory, non-autoimmune CNS disease that may occur in the subject or prevent its occurrence.

In one embodiment, the subject is human and the risk is associated with a genetic predisposition to a chronic medical condition.

In another embodiment, the disease is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, glaucoma, dementia, neuropathy, stroke, and brain tumor.

In a further embodiment, the route of administration is oral, intravenous, intramuscular, subcutaneous, intraperitoneal, transdermal, nasal or rectal.

The subject invention additionally includes a method of inhibiting the activity of a matrix metalloproteinase comprising contacting the matrix metalloproteinase with glatiramer acetate (Copolymer 1).

In one embodiment, the matrix metalloproteinase is MMP-9.

In another embodiment, the matrix metalloproteinase is in a subject.

In one embodiment, the subject is human.

The subject invention further provides a method of suppressing the production of a cytokine by activated T lymphocytes comprising contacting the activated T lymphocytes with glatiramer acetate (Copolymer 1) in an amount necessary to suppress the production of the cytokine.

In one embodiment, the cytokine is IL-1.

In another embodiment, the cytokine is IL-4.

In a further embodiment, the cytokine is IL-6.

In yet ancther embodiment, the cytokine is IL-10.

In a further embodiment, the cytokine is IL-12.

In another embodiment, the cytokine is IL-13.

In a further embodiment, the cytokine is TNF-a.

In one embodiment, the cytokine is in a subject.

In another embodiment, the subject is human.

The subject invention also contains the use of glatiramer acetate (Copolymer 1) in the preparation of a pharmaceutical composition for the treatment of an inflammatory, non-autoimmune central nervous system (CNS) disease, or alleviation of the symptoms of such a disease, wherein said pharmaceutical composition is

administered to the subject in an amount and for a duration of time effective to treat the inflammatory, non-autoimmune CNS disease in a mammalian subject.

In one embodiment of the use, the mammalian subject is human.

In another embodiment of the use, the disease is Alzheimer's Disease.

In a further embodiment of the use, the disease is Parkinson's Disease.

In yet another embodiment of the use, the disease is HIV encephalopathy.

In a further embodiment of the use, the disease is a brain tumor.

In another embodiment of the use, the disease is glaucoma.

In a further embodiment of the use, the disease is neuropathy or dementia.

In another embodiment of the use, the disease is a CNS infection.

In one embodiment of the use, the CNS infection is a bacterial infection.

In another embodiment of the use, the bacterial infection is meningitis.

In a further embodiment of the use, the disease results from stroke.

In yet another embodiment of the use, the disease results from head trauma.

The subject invention encompasses embodiments of the use wherein the route of administration is oral, intravenous, intramuscular, subcutaneous, intraperitoneal, transdermal, nasal or rectal. The preferred routes of administration are oral and subcutaneous injection.

In one embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered ranges from about .1 mg to about 1000 mg.

In another embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 1 mg to about 100 mg.

In a further embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 5 mg to about 50 mg.

In yet another embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 10 mg to about 30 mg.

In one embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered is about 20 mg.

In another embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.05 mg/kg of body weight to about 50 mg/kg of body weight.

In a further embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered ranges from about .1 mg/kg of body weight to about 10 mg/kg of body weight.

In an additional embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.1 mg/kg of body weight to about 1.0 mg/kg of body weight.

In another embodiment of the use, the dose of glatiramer acetate (Copolymer 1) administered is about 0.3 mg/kg of body weight.

In one embodiment of the use, the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every 30 days to about once every day. In a preferred embodiment of the use, the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every 7 days to about once every day. In a more preferred embodiment of the use, the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every day.

In another embodiment of the use, the glatiramer acetate (Copolymer 1) is administered as part of a therapeutic regimen during which a cytokine antagonist is also administered to the subject.

The subject invention also concerns the use of glatiramer acetate (Copolymer 1) in the preparation of a pharmaceutical composition for treating a mammalian subject at risk of developing an inflammatory, non-autoimmune CNS disease, wherein said pharmaceutical composition is administered to the subject in an amount and for a duration of time effective to minimize the severity of the inflammatory, non-autoimmune CNS disease that may occur in the subject or prevent its occurrence.

In one embodiment of the use, the subject is human and the risk is associated with a genetic predisposition to a chronic medical condition.

In another embodiment of the use, the disease is selected from the group consisting of Alzheimer's Disease, Parkinson's Disease, glaucoma, dementia, neuropathy, stroke, and brain tumor.

In a further embodiment of the use, the route of administration is oral, intravenous, intramuscular, subcutaneous,

intraperitoneal, transdermal, nasal or rectal.

The subject invention additionally includes the use of glatiramer acetate (Copolymer 1) in the preparation of a pharmaceutical composition for inhibiting the activity of a matrix metalloproteinase.

In one embodiment of the use, the matrix metalloproteinase is MMP-9.

In another embodiment of the use, the matrix metalloproteinase is in a subject.

In one embodiment of the use, the subject is human.

The subject invention further provides the use of glatiramer acetate (Copolymer 1) in the preparation of a pharamcutical composition for suppressing the production of a cytokine by activated T lymphocytes.

In one embodiment of the use, the cytokine is IL-1.

In another embodiment of the use, the cytokine is IL-4.

In a further embodiment of the use, the cytokine is IL-6.

In yet another embodiment of the use, the cytokine is IL-10.

In a further embodiment of the use, the cytokine is IL-12.

In another embodiment of the use, the cytokine is IL-13.

In a further embodiment of the use, the cytokine is TNF-a.

In one embodiment of the use, the cytokine is in a subject.

In anctner embodiment of the use, the subject is human.

COPAXONE® is the brand name for glatiramer acetate (formerly known as copolymer-1). Glatiramer acetate, the active ingredient of COPAXONE®, consists of the acetate salts of synthetic polypeptides, containing four naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine, and L-lysine with an average molar fraction of 0.141, 0.427, 0.095, and 0.338, respectively. The average molecular weight of glatiramer acetate is 4,700 - 11,000 daltons. Chemically, glatiramer acetate is designated L-glutamic acid polymer with L-alanine, L-lysine and L-tyrosine, acetate (salt). Its structural formula is:

$$(Glu, Ala, Lys, Tyr)_x \cdot xCH_3COOH$$
$$(C_5H_9NO_4 \cdot C_3H_7NO_2 \cdot C_6H_{14}N_2O_2 \cdot C_9H_{11}NO_3)_x \cdot xC_2H_4O_2$$
CAS - 147245-92-9

(92).

Copolymer 1 (Cop-1) can be formulated into pharmaceutical compositions containing a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, adjuvants, suspending agents, emulsifying agents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, sweeteners, flavor enhancers and the like. The pharmaceutically acceptable carriers may be prepared from a wide range of materials including, but not limited to, flavoring agents, sweetening agents and miscellaneous materials such as buffers and absorbents that may be needed in order to prepare a particular oral therapeutic composition. The use of such media and agents with pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated.

Copolymer 1 can be formulated into any form known in the art using procedures available to one of skill in the art. Copolymer

1 may be mixed with other food forms and consumed in solid, semi-solid, suspension or emulsion form. In one embodiment, the composition is formulated into a capsule or tablet using techniques available to one of skill in the art. However, the present compositions may also be formulated in another convenient form, such as an injectable solution or suspension, a spray solution or suspension, a lotion, a gum, a lozenge, a food or snack item. Food, snack, gum or lozenge items can include any ingestible ingredient, including sweeteners, flavorings, oils, starches, proteins, fruits or fruit extracts, vegetables or vegetable extracts, grains, animal fats or proteins. Thus, the present compositions can be formulated into cereals, snack items such as chips, bars, gum drops, chewable candies or slowly dissolving lozenges. Copolymer 1 can also be administered as dry powder or metered dose of solution by inhalation, or nose-drops and nasal sprays, using appropriate formulations and metered dosing units.

One of skill in the art can readily substitute structurally-related amino acids for Copolymer 1 without deviating from the spirit of the invention. The present invention includes polypeptides and peptides which contain amino acids that are structurally related to tyrosine, glutamic acid, alanine or lysine and possess the ability to stimulate polyclonal antibody production upon introduction. Such substitutions retain substantially equivalent biological activity in their ability to suppress or alleviate the symptoms of the CNS disease. These substitutions are structurally-related amino acid substitutions, including those amino acids which have about the same charge, hydrophobicity and size as tyrosine, glutamic acid, alanine or lysine. For example lysine is structurally-related to arginine and histidine; glutamic acid is structurally-related to aspartic acid; tyrosine is structurally-related to serine, threonine, phenylalanine and tryptophan; and alanine is structurally-related to valine, leucine and isoleucine. These and other conservative substitutions, such as structurally-related synthetic amino

acids, are contemplated by the present invention.

Moreover, Copolymer 1 can be composed of l-or d- amino acids. As is known by one of skill in the art, l-amino acids occur in most natural proteins. However, d- amino acids are commerically available and can be substituted for some or all of the amino acids used to make Copolymer 1. The present invention contemplates Copolymer 1 consisting essentially of l-amino acids, as well as Copolymer 1 consisting essentially of d-amino acids.

Experimental Details

Procedure

PBMC Isolation

Heparinized blood was collected from normal volunteers, and subjected to Ficoll-Hypaque (Pharmacia Biotech, Mississauga, Ontario) centrifugation to obtain peripheral blood mononuclear cells (PBMNCs) as described previously (15). After two washes, cells were suspended at a density of 1-2 million/ml in horizontal T-25 flasks (Nunc, Becton Dickinson, Mississauga, Ontario) in the serum free medium, AIM-V (GIBCO/BRL), to which 1 ng/ml of an anti-CD3 antibody (OKT3, compliments of Jack Antel, Montreal, Canada) was added. Three hours after the anti-CD3 addition, the T-25 flasks were stood upright from their horizontal position in order to kill monocytes that had adhered. Floating cells, which were mostly lymphocytes, were left for a period of 72 hours at 37°C. Flow cytometry analysis of the MNC population at this 72 hour period indicated that CD3+ cells constituted about 90% of the total cell population, with approximately 60% CD4+ and 30% CD8+ ratio. B lymphocytes (CD19+) and NK cells (CD56+) consisted of 5-6% of the total MNC population, and no monocytes (CD14+) were detected. Henceforth, given that the majority of cells in the MNC population are T lymphocytes, these will be referred to as T lymphocytes.

Copolymer 1 Treatment

Copolymer 1 (1-50 μg/ml) diluted in phosphate-buffered saline, was added to cultures 3 hours after the initiation of CD3 ligation, at the time that the T-25 flasks were altered from the horizontal to upright positions. Cells were left for 69h at 37°C, then collected, counted and resuspended in fresh AIM-V at density of 500,000 cells per ml. Following a second treatment with Copolymer 1, cells were left at 37°C for an additional 3h. Thereafter, 500μl (250,000 cells) of the suspension was taken for migration assays described below. Alternatively, 100 μl (50,000 cells) of cell suspension was added to each individual well of a 96-well plate already containing microglia or U937 monocytoid cells (see below). The experiments involved 72h treatment of T lymphocytes with Copolymer 1, administered at 2 time points. The purity of T lymphocytes after 72h of Copolymer 1 treatment was not different from that of non-treated controls. In some experiments, recombinant IFNβ-1b was used as a positive control.

Monocyte-Enriched Culture

To produce monocyte-enriched cultures, 2 x 10^5 PBMC were suspended in 100 μl of AIM-V medium and placed into each well of a 96-well plate. One hour after, floating cells were removed to leave behind adherent monocytes. Fresh 100 μl of AIM-V was added per well and cultures were kept at 37°C. Copolymer 1 was added directly to cells in the 96-well plate.

MMPs Assay

To address whether the production of MMPs by cells was affected by Copolymer 1, the conditioned medium of cells was used, since MMPs are secreted enzymes. Essentially, for floating T lymphocytes contained within the T-25 flasks, 1 ml of conditioned medium was siphoned off and microfuged for 1 min to remove cells. Supernatant was collected, mixed in a 3.1 ratio (v/v) with 4x gel-loading SDS buffer, and used in zymographic assays. Similarly, for monocytes in wells of 96-well plates, conditioned medium was collected, microfuged, and mixed with 4x gel-loading SDS buffer.

Zymography is essentially SDS-PAGE except that gelatin is also added to the gel (77, 82). After proteins were resolved based on molecular weight in SDS-PAGE, the gel was washed in a Triton X-100 buffer in order to remove SDS which thus allows protein renaturation. In a calcium-containing "reaction buffer" (77), gelatinases (MMP-2 and -9) degraded gelatin in their immediate vicinity (other members of MMPs would also be detected if present in high amounts). Following staining in Coommasie blue, which binds all proteins, and destaining , areas containing gelatinases appeared as clear bands against a dark background, since the gelatin in its immediate vicinity has been degraded. The size of the MMP bands was a reflection of the amount of MMP that was produced by cells, and this was documented by NIH image analysis software. The molecular weight of the migrated bands revealed the MMP species, which confirmed previous Western blot analyses and immunoprecipitations (77, 82).

The analysis of cell conditioned medium in zymography is a reflection of the amount of MMPs that was produced by the particular culture. To determine whether Copolymer 1 was an inhibitor of the activity of MMPs, independent from its effect on levels of MMP protein, supernatants from BHK cells, a standard source of MMPs in many laboratories, or supernatants from T lymphocytes were resolved by electrophoresis in SDS-PAGE containing gelatin. During the development of the gelatinolytic activity, Copolymer 1 was added to the Triton-100 wash, and the "reaction buffer", described above. An inhibitor of MMP activity prevents gelatin degradation by gelatinases and the resultant size of the band of gelatinases would be smaller as compared to that obtained in the absence of inhibitors.

Migration Assay

To address the migration capacity of T lymphocytes, 2 x 10^5 T lymphocytes in 500 μl of 2.5% fetal calf serum (FCS) containing AIM-V were seeded into the top compartment of a Boyden chamber (Collaborative Biomedical Products, Bedford, Maryland). This

chamber consists of two compartments separated by a polycarbonate membrane filter (9 mm in diameter, with 5 μm pores) precoated on its upper surface with fibronectin. Fibronectin was employed to simulate the basal lamina.

The bottom compartment of the migration chamber contained 10% FCS-supplemented AIM-V; the higher concentration of FCS in the bottom chamber served as a directional gradient (77). After 5h at 37°C, the content of the lower chamber was collected, and the number of migrated T lymphocytes was counted with the Coulter Counter Z1.

Microglial Cells

Fetal human microglia were isolated from brains obtained at legal and therapeutic abortions using a protocol described by Lee et al. (41). Specimens ranged in gestational age from 14 to 20 weeks. 2.5 X 10^4 microglia of over 95% purity were plated per well of a 96 well plate. Microglia culture medium was minimum essential medium supplemented with 5% FCS, 0.1% dextrose. For co-cultures, 100μl containing 50,000 T lymphocytes in AIM-V (as described above) was added to individual wells of a 96-well plate already containing 25,000 microglia (or U937 monocytoid cells - see below) in microglia culture medium. Twenty four hours after, conditioned medium was collected for cytokine quantifications by ELISA.

Adult human microglia were isolated from the resected brain tissue of patients undergoing surgical resection to treat intractable epilepsy. Microglia of over 95% purity was obtained using a previously detailed protocol (90,. Cells were used for interactions with T lymphocytes in a manner identical to that described for their fetal counter parts.

A human promonocytoid cell line, U937, was also employed. Members of this cell line become microglia-like, as assessed by morphology and expression of cell surface molecules, when treated

sequentially with 50 ng/ml of a protein kinase C activator, phorbol-12-myristate-13-acetate (PMA) (time 0-48) and 100 U/ml interferon-γ (IFNγ) (from 48-72h).

Cells were used 1-3 days after the IFNγ treatment. As with microglia, 50,000 T lymphocytes in AIM-V was added to individual wells of a 96-well plate already containing 25,000 PMA/IFNγ-treated U937 cells, and conditioned medium was collected after 24h of co-cultures.

Cytokine and Chemokine Assays

Cytokine protein levels in the conditioned medium of microglia-T lymphocyte co-cultures were measured using enzyme-linked immunoabsorbent assay (ELISA) kits bought from BioSource International (Montreal, Canada).

Chemokine and chemokine receptor expression are currently recognized to be important mechanisms that regulate the influx of leukocytes into the CNS. Notable is the up-regulation of the chemokine IP-10 and its receptor CXCR3 in the lesions of multiple sclerosis patients (74). Flow cytometry was employed to determine whether Copolymer 1 affected the expression of the CXCR3 receptor. Activated T lymphocytes or U937 cells were treated with 25 μg/ml of Copolymer 1 for 1-3 days.

Results

Effect of Copolymer 1 on MMPs

Activated T lymphocytes secreted MMP-9 into the culture medium which peaked between 2 - 3d of anti-CD3 ligation (Fig. 1). Treatment of anti-CD3 activated T lymphocytes with Copolymer 1 (25 μg/ml, for 1,2 or 3d) did not affect their production of MMP-9 as compared to controls (Fig. 1) and neither did a higher concentration of Copolymer 1 (50 μg/ml). Monocyte production of MMP-9 was also unaffected by Copolymer 1. In contrast, and in agreement with a previous report (77), T lymphocytes treated with IFNβ-1b produced less MMP-9 when compared to control T

lymphocytes (Fig. 1).

Figure 2 demonstrates that Copolymer 1 exhibited MMP inhibitory activity, since the size of the gelatinase band (MMP-9) was dose-dependently reduced by Copolymer 1 as compared to controls. In contrast, IFNβ-1b, which inhibits the production of MMP-9 (Fig. 1), is not a direct inhibitor of MMP enzyme activity. These results indicate that Copolymer 1 is an inhibitor of MMP enzyme activity.

Effect of Copolymer 1 on Cell Migration

Figure 3-A shows that the Copolymer 1 did not inhibit the migration of T lymphocytes when the T lymphocytes were pre-treated for 1,2 or 3 days with Copolymer 1 (25 μg/ml). Similarly, pretreatment of T lymphocytes for 3 days with various concentrations of Copolymer 1 (1 to 50 μg/ml) did not affect the trans-migration of activated T lymphocytes (Fig. 3-B).

In a separate series of experiments, 10 ng/ml monocyte chemoattractant protein (MCP)-1 or MCP-3 was placed in the bottom chamber to serve as a chemotactic signal. Under these conditions, the migration rate of glatiramer pre-treated T lymphocytes also did not differ from that of controls.

Effect of Copolymer 1 on T Lymphocyte/Microglia Cultures

Neither PMA/IFNγ-treated U937 cells nor anti-CD3-activated T lymphocytes produced detectable levels of TNF-α in isolation. However, IL-10 was detected in U937 but not in T lymphocytes conditioned medium (Fig. 4). In co-culture for 24h, substantial increases in levels of TNF-α and IL-10 were obtained. Treatment of T lymphocytes with Copolymer 1 prior to their encounter with PMA/IFNγ-treated U937 cells reduced, in a dose-dependent manner, the production of TNF-α and IL-10 (Fig. 4). The effect of Copolymer 1 appears to be principally on T lymphocytes since the pretreatment of PMA/IFNγ-treated U937 cells with Copolymer 1 did not influence cytokine production in subsequent T lymphocyte-U937

interactions.

Interactions between activated T lymphocyte and PMA/IFNγ-treated U937 also led to the upregulation of two Th2-like anti-inflammatory cytokines, IL-4 and IL-13 (Fig. 5), although the amounts produced were about a log fold lower than those of IL-10 or TNF-α. This production of IL-4 and IL-13 was significantly reduced by Copolymer 1 in co-cultures of T lymphocytes with PMA/IFNγ-treated U937, and this decrease was also observed for the pro-inflammatory cytokine, IL-12 (Fig. 5). In summary, Copolymer 1 pretreatment (72 h) of anti-CD3 ligated T lymphocytes results in the suppression of all inducible cytokines that were examined in the T lymphocyte-U937 interactions.

The effect of Copolymer 1 in reducing cytokine production in T lymphocyte - U937 co-cultures is not a result of a decrease in the proliferation of T lymphocytes, since Copolymer 1 does not decrease the number or size of T lymphocyte aggregates that form following anti-CD3 treatment, indicating that it does not affect the proliferation of T lymphocytes in any significant manner. Indeed, when the total number of cells was counted after 72h of Copolymer 1, cell numbers were comparable in the control (24 ± 2 x 10^3) versus Copolymer 1-treated (5, 25 and 50 μg/ml) groups (26 ± 1, 26 ± 2 and 22 ± 1, respectively, x 10^3). Equal numbers of T lymphocytes were added to microglia or U937 cells in all test situations.

Fetal human microglia in isolation do not secrete detectable amounts of IL-10 or TNF-α into the culture medium; thus, it appears that these cells are similar to their adult counterparts (14, 15). In co-culture with activated T lymphocytes, significant amounts of IL-10 and TNF-α were produced (139 ± 4 and 1068 ± 68 ng/ml, respectively, Fig. 6). With Copolymer 1 pre-treated T lymphocytes, the resultant IL-10 and TNF-α in T lymphocyte - microglia co-cultures were significantly reduced (Fig. 6). Indeed, the reduction by Copolymer 1 occurred in a

dose-dependent manner (Fig. 6).

The interaction of T lymphocytes with adult microglia from human neural cells led to the upregulation of IL-10 and TNF-α from previously undetectable levels, and this was also the case for IL-1β. IL-6 was constitutively expressed at high levels by microglia (Fig. 7). The pretreatment of T lymphocytes with Copolymer 1 resulted in a dose-dependent inhibition of the inducible cytokines (IL-1β, IL-10 and TNF-α). Similarly, Copolymer 1 decreased the expression of the constitutive cytokine, IL-6 (Fig. 7).

It should be noted that T lymphocytes have to be activated with anti-CD3 antibody since co-cultures of unactivated T lymphocytes (even in the presence of 50 U/ml IL-2) with microglia did not result in increased production of TNF-α. Moreover, it is necessary for T lymphocytes to be pretreated with Copolymer 1 since its reducing effect on cytokine production does not occur if it is added at the time of co-culture.

Figure 8 demonstrates that when adult microglia encounter activated T lymphocytes in vitro, the morphology of microglia transforms from a ramified/bipolar morphology to an ameoboid rounded form (14). However, when T lymphocytes were pretreated with Copolymer 1, the morphological transformation of microglia in T lymphocyte - microglia co-culture was attenuated. This was also the case for fetal human microglia or PMA/IFNγ-treated U937 cells in co-culture with activated T lymphocytes. Overall, the lack of a morphological transformation of microglia is another indication that Copolymer 1-pretreatment of T lymphocytes resulted in their decreased ability to interact with microglia.

Effect of Copolymer 1 on Chemokines

Figure 9 indicates that Copolymer 1 treatment of activated T lymphocytes did not reduce their expression of CXCR3 after 1-3 days of treatment; this negative result was also the case for the

U937 cell line (Fig. 10). CXCR4 expression was also evaluated and found to be unresponsive to Copolymer 1 treatment.

Discussion

Copolymer 1, or glatiramer acetate (GA), is a heterogeneous mixture of synthetic random linear copolymers of tyrosine, alanine, glutamic acid and lysine. This drug is effective in treating multiple sclerosis.

The mechanism of action of Copolymer 1 in multiple sclerosis is based on its capacity to suppress an immune response, which specifically affects clinical manifestations of the disease. The subject invention demonstrates that Copolymer 1 has additional biological activities, such as CNS anti-inflammation and remyelination. These findings raise the possibility of extending the potential therapeutic effect of Copolymer 1 in additional indications other than multiple sclerosis.

The capability of Copolymer 1 to bind to MHC class II molecules of various genetic backgrounds, resulting in the inhibition of T-cell responses, led to the hypothesis that it may act as a general immunosuppressor.

Results from in vivo animal models and from Copolymer 1 treated multiple sclerosis patients suggest that, in most cases, administration of Copolymer 1 in a dosing protocol that affects multiple sclerosis or EAE does not result in non-specific immunosuppression. Rather, the beneficial therapeutic effect of Copolymer 1 in multiple sclerosis and EAE is probably mediated by antigen-specific suppressor T cells.

On the other hand, administration of Copolymer 1 using different protocols (higher doses and/or more frequent administration) was shown to interfere with alloreactivity in vivo, prevent experimental GVHD and have a beneficial effect on skin and thyroid engrafment in mice models. These effects of Copolymer

1 are most probably mediated by its ability to compete with antigens for their presentation by MHC molecules on antigen presenting cells, which has been shown to be dose-dependent. Another proof for this postulate is the fact that D- Copolymer 1 - is very active in the mouse GVHD model. In fact - it is 10 times more active than L-Copolymer 1. Thus, it is reasonable to predict that using doses of Copolymer 1 higher than those used for the treatment of multiple sclerosis, will enable it to work as a general immunosuppressor, and thus to be clinically relevant in indications other than multiple sclerosis.

In view of the avid binding of Copolymer 1 to class II molecules on antigen presenting cells, studies were designed to examine whether it can interfere with T-cell mediated immune responses. Copolymer 1 reportedly inhibited in vitro T-cell responses to MBP in a dose-dependent manner (5, 11, 58, 60, 78, 80). Due to the cross-reactivity between Copolymer 1 and MBP, this effect can be attributed not only to competition for the binding to MHC class II molecules, but also to mechanisms related to T-cell recognition (1, 73). However, the inhibitory capacity of Copolymer 1 was shown to extend also to non cross-reactive T-cell responses (7, 60, 79, 83). In this case, the inhibitory effect is most probably due to competition for binding to MHC class II molecules.

Copolymer 1 binds to murine and human antigen presenting cells irrespective of their MHC restriction (promiscuous binding) in a dose-dependent manner (29, 30). Binding of Copolymer 1 to MHC class II molecules was demonstrated by its specific inhibition with anti-class II antibodies (30). Evidence for the direct interaction of Copolymer 1 with various purified HLA-DR molecules has been recently reported (28). Furthermore, Copolymer 1 was shown to compete and even displace antigens already bound to MHC class II molecules (30, 31, and 79). Binding to MHC class II molecules is rapid and efficient (30), and no processing seems to be required for this interaction (29).

A recent study observed that lymphocytes isolated from relapsing-remitting multiple sclerosis patients on Copolymer 1 were significantly reduced in their capacity to transmigrate across a fibronectin barrier, when compared to cells from untreated multiple sclerosis patients (59). Fibronectin was used as a model of basal lamina. Since the transmigration of leukocytes across the fibronectin barrier is correlated with their expression of matrix metalloproteineses (42, 77, 88), it was of interest to assess the effect of Copolymer 1 on MMPs. The subject invention shows that Copolymer 1 inhibits MMP-9 enzyme activity, but does not decrease the production of MMP-9 by T lymphocytes or monocytes. This inhibition does not alter lymphocyte transmigration.

The findings that Copolymer 1 is an inhibitor of MMP enzyme activity is relevant to MMP-mediated effects that are unrelated to leukocyte trafficking. For instance, many cytokines (e.g. TNF-α and TGF-α), cytokine receptors (e.g., TNFRs, IL-6Rα) and adhesion molecules (e.g., L-selectin, VCAM) are synthesized in pro-forms that require proteolytic processing to generate the active agent. While the identity of these "convertases" or "shedases" remains unresolved, and likely are members of another group of metalloproteinases (adamalysins), MMPs have the capacity to convert promolecules (e.g. pro-TNF-α) to their active forms (e.g. TNF-α), hence producing a proinflammatory environment within the CNS (91). The subject invention demonstrates the inhibition of MMP enzyme activity by Copolymer 1, which may decrease the conversion of procytokines to cytokines, resulting in a non-inflammatory milieu (Fig. 2).

It has been well documented that many cells are dependent on attachment to extracellular matrix (ECM) molecules for survival. Detachment of these cells from their extracellular matrix substrate results in their apoptosis, a phenomenon that has been refered to as "anoikis" (64, 65). Because MMPs are the physiological mediators of extracellular matrix turnover, their

aberrant expression and activity can disrupt the integrity of the extracellular matrix, and thus result in altered cell adhesion and death (85). By acting as an inhibitor of MMP activity, glatiramer acetate (Copolymer 1) may prevent the disruption of the extracellular matrix, and prevent the detachment of cells from the extacellular matrix. A consequence is thus decreased neural cell death and thereby the progression of the disease is slowed. MMPs are also implicated in cell-ECM interactions that govern processes as diverse as cellular differentiation, migration and inflammation. In the developing nervous system, MMP family members regulate angiogenesis, extension of neuronal growth cones and process formation by oligodendrocytes (54).

MMPs may also suppress the production of anti-inflammatory cytokines, as suggested by the findings that the treatment of EAE animals with MMP inhibitors led to the increase of the anti-inflammatory cytokine, IL-4, within the CNS (43); the mechanism of this activity remains unexplained. Other consequences of aberrant MMP expression within the CNS can include a direct role in myelin destruction (48) and the generation of encephalitogenic fragments of myelin which leads to the propagation of inflammation (16, 56). The subject finding that Copolymer 1 inhibits MMP enzyme activity could be relevant to these observations and lead to an anti-inflammatory milieu in the CNS.

The encounter of T lymphocytes with microglia is a significant source of numerous cytokines. Copolymer 1 pretreatment of T lymphocytes resulted in a substantial diminution of all cytokines tested in T lymphocyte - microglia (or U937 cell) interactions.

Immune deviation is a concept that has gained attention in recent years. This concept has its origin in the observation that uncommitted T lymphocytes can differentiate along either the Th1 route, with the production of pro-inflammatory cytokines such as IFN-γ, IL-12 or TNF-α, or into the Th2 pathway with the production of Th2-like anti-inflammatory cytokines, including IL-

4, IL-10, IL-13 or transforming growth factor-βs (TGF-βs); cells that produce TGF-βs have also been referred to as Th3 cells. Susceptibility to certain diseases has been attributed to a predominant Th1 or Th2 response (46).

The disclosed experiments, using an antigen independent system, suggest that Copolymer 1 does not have a preferential effect on Th1 or Th2 type cytokines within the CNS, since all cytokines, including TNF-α, IL-4, IL-6, IL-10, Il-12 and IL-13, are suppressed in the T lymphocyte - microglia interactions, creating a non-inflammatory milieu.

Although Copolymer 1 lessened the secretion of all cytokines tested (pro-inflammatory and anti-inflammatory), treatment of patients with CNS conditions should result in lessened T lymphocyte-mediated inflammation. The decreased inflammation should lessen the severity of the disease. Neuronal and axonal integrity should also improve in inflammatory CNS disorders since inflammation is also associated with the destruction of neurons and axons. Furthermore, individuals at risk for developing CNS diseases could be treated with Copolymer 1 to prevent the onset of the disease or lessen its severity. In addition, the lessened production of TNF-α, a cytokine that is capable of killing oligodendrocytes (25, 44, 45, 69), would also decrease the degree of oligodendrocyte loss and demyelination in the CNS of patients on Copolymer 1. Additionally, Copolymer 1, by inhibition of cytokine secretion, may decrease the generation of free radicals, which cause cellular damage and destruction. Therefore, Copolymer 1 would not only alleviate the symptoms associated with CNS inflammation, but also slow the progression of the CNS disease itself.

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What is claimed is:

1. A method of treating a mammalian subject having an inflammatory, non-autoimmune central nervous system (CNS) disease, or alleviating the symptoms of such a disease, comprising administering glatiramer acetate (Copolymer 1) to the subject in an amount and for a duration of time effective to treat the inflammatory, non-autoimmune CNS disease.

2. The method of claim 1, wherein the mammalian subject is human.

3. The method of claim 1, wherein the disease is Alzheimer's Disease.

4. The method of claim 1, wherein the disease is Parkinson's Disease.

5. The method of claim 1, wherein the disease is HIV encephalopathy.

6. The method of claim 1, wherein the disease is a brain tumor.

7. The method of claim 1, wherein the disease is glaucoma.

8. The method of claim 1, wherein the disease is neuropathy or dementia.

9. The method of claim 1, wherein the disease is a CNS infection.

10. The method of claim 9, wherein the CNS infection is a bacterial infection.

11. The method of claim 10, wherein the bacterial infection is meningitis.

12. The method of claim 1, wherein the disease results from stroke.

13. The method of claim 1, wherein the disease results from head trauma.

14. The method of claim 1, wherein the route of administration is oral, intravenous, intramuscular, subcutaneous, intraperitoneal, transdermal, nasal or rectal.

15. The method of claim 14, wherein the route of administration is oral.

16. The method of claim 14, wherein the route of administration is subcutaneous injection.

17. The method of claim 14, wherein the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.05 mg/kg of body weight to about 50 mg/kg of body weight.

18. The method of claim 17, wherein the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.1 mg/kg of body weight to about 10 mg/kg of body weight.

19. The method of claim 18, wherein the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.1 mg/kg of body weight to about 1.0 mg/kg of body weight.

20. The method of claim 19, wherein the dose of glatiramer acetate (Copolymer 1) administered is about 0.3 mg/kg of body weight.

21. The method of claim 14, wherein the dose of glatiramer acetate (Copolymer 1) administered ranges from about 0.1 mg

to abcut 1000 mg.

22. The method of claim 21, wherein the dose of glatiramer acetate (Copolymer 1) administered ranges from about 1.0 mg to about 100 mg.

23. The method of claim 22, wherein the dose of glatiramer acetate (Copolymer 1) administered ranges from about 10 mg to about 30 mg.

24. The method of claim 23, wherein the dose of glatiramer acetate (Copolymer 1) administered is about 20 mg.

25. The method of claim 14, wherein the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every 30 days to about once every day.

26. The method of claim 25, wherein the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every 7 days to about once every day.

27. The method of claim 26, wherein the dose of glatiramer acetate (Copolymer 1) is administered at a frequency of about once every day.

28. The method of claim 1, wherein the glatiramer acetate (Copolymer 1) is administered as part of a therapeutic regimen during which a cytokine antagonist is also administered to the subject.

29. A method of treating a mammalian subject at risk of developing an inflammatory, non-autoimmune CNS disease comprising administering glatiramer acetate (Copolymer 1) to the subject in an amount and for a duration of time effective to minimize the severity of the inflammatory, non-autoimmune CNS disease that may occur in the subject or prevent its occurrence.

30. The method of claim 29, wherein the subject is human and the risk is associated with a genetic predisposition to a chronic medical condition.

31. The method of claim 29, wherein the disease is selected from the group consisting of Alzheimer's disease, Parkinson's disease, glaucoma, dementia, neuropathy, stroke, and brain tumor.

32. The method of claim 29, wherein the route of administration is oral, intravenous, intramuscular, subcutaneous, intraperitoneal, transdermal, nasal or rectal.

33. A method of inhibiting the activity of a matrix metalloproteinase comprising contacting the matrix metalloproteinase with glatiramer acetate (Copolymer 1).

34. The method of claim 33, wherein the matrix metalloproteinase is MMP-9.

35. The method of claim 34, wherein the matrix metalloproteinase is in a subject.

36. The method of claim 35, wherein the subject is human.

37. A method of suppressing the production of a cytokine by activated T lymphocytes comprising contacting the activated T lymphocytes with glatiramer acetate (Copolymer 1) in an amount necessary to suppress cytokine production.

38. The method of claim 37, wherein the cytokine is IL-1.

39. The method of claim 37, wherein the cytokine is IL-4.

40. The method of claim 37, wherein the cytokine is IL-6.

41. The method of claim 37, wherein the cytokine is IL-10.

42. The method of claim 37, wherein the cytokine is IL-12.

43. The method of claim 37, wherein the cytokine is IL-13.

44. The method of claim 37, wherein the cytokine is TNF-a.

45. The method of claim 37, wherein the cytokine is in a subject.

46. The method of claim 45, wherein the subject is human.

The Use of Glatiramer Acetate (Copolymer 1) in the Treatment of Central Nervous System Disorders

Abstract of the Disclosure

The present invention involves the administration of Copolymer 1 (glatiramer acetate) to treat inflammatory, non-autoimmune central nervous system (CNS) diseases, alleviate the symptoms thereof, inhibit the activity of matrix metalloproteinases and suppress cytokine production by T lymphocytes.

FIGURE 1

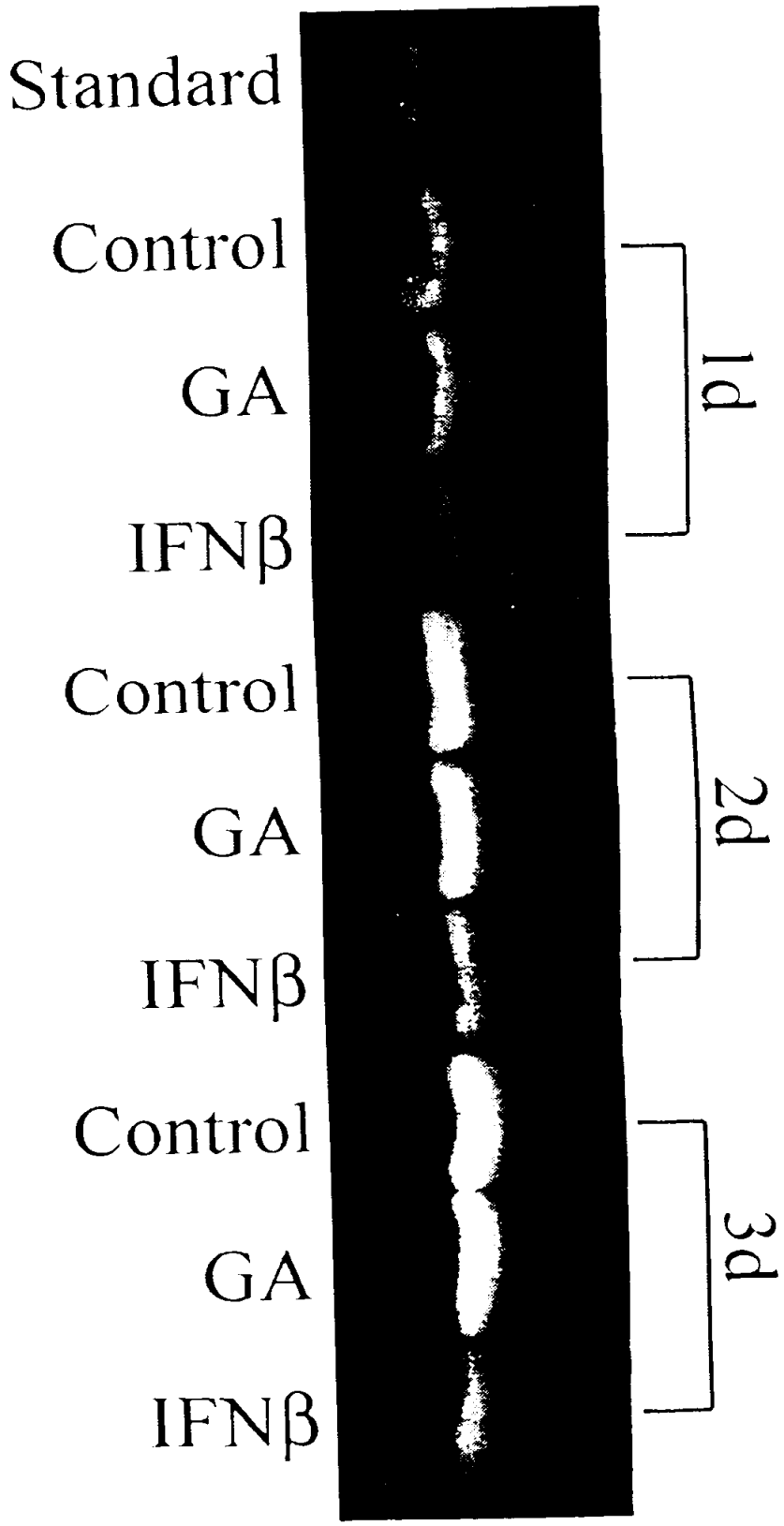

Standard
Control
GA
IFNβ
Control
GA
IFNβ
Control
GA
IFNβ
1d
2d
3d

FIGURE 2

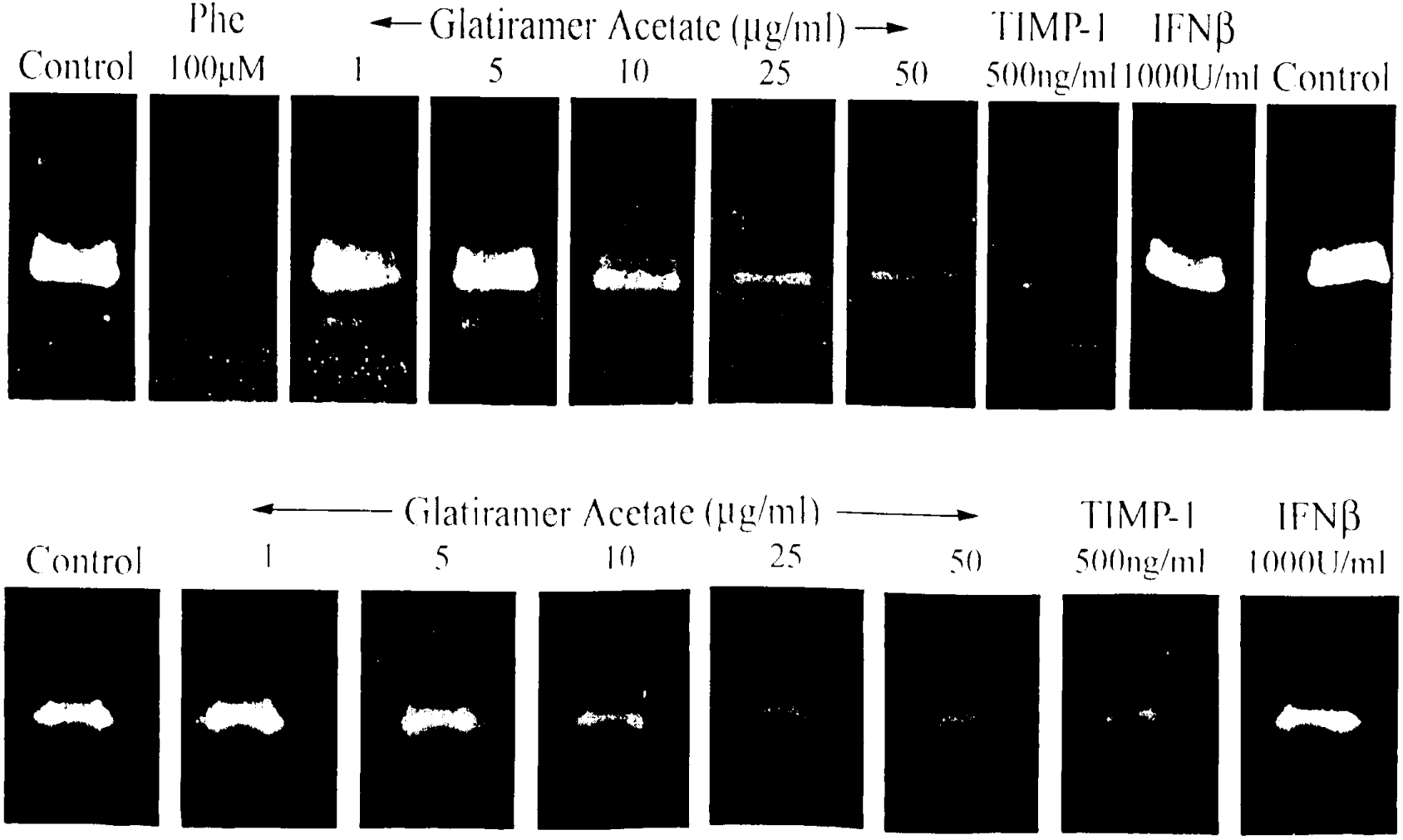

Phe
← Glatiramer Acetate (µg/ml) →
TIMP-1
IFNβ
Control
100µM
1
5
10
25
50
500ng/ml
1000U/ml
Control
← Glatiramer Acetate (µg/ml) →
TIMP-1
IFNβ
Control
1
5
10
25
50
500ng/ml
1000U/ml

FIGURE 3

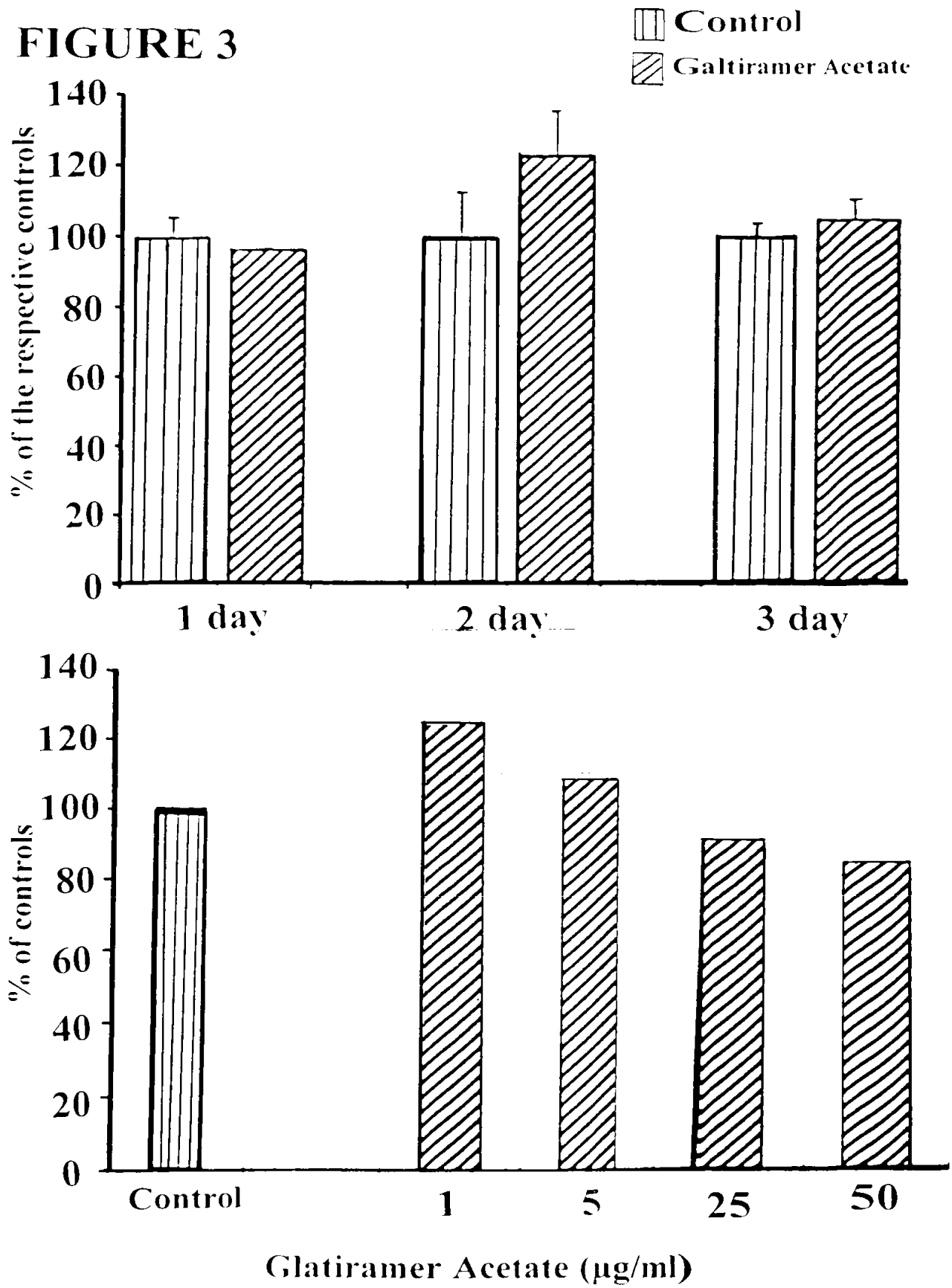

Control
Galtiramer Acetate
% of the respective controls
140
120
100
80
60
40
20
0
1 day
2 day
3 day
% of controls
140
120
100
80
60
40
20
0
Control
1
5
25
50
Glatiramer Acetate (µg/ml)

FIGURE 4

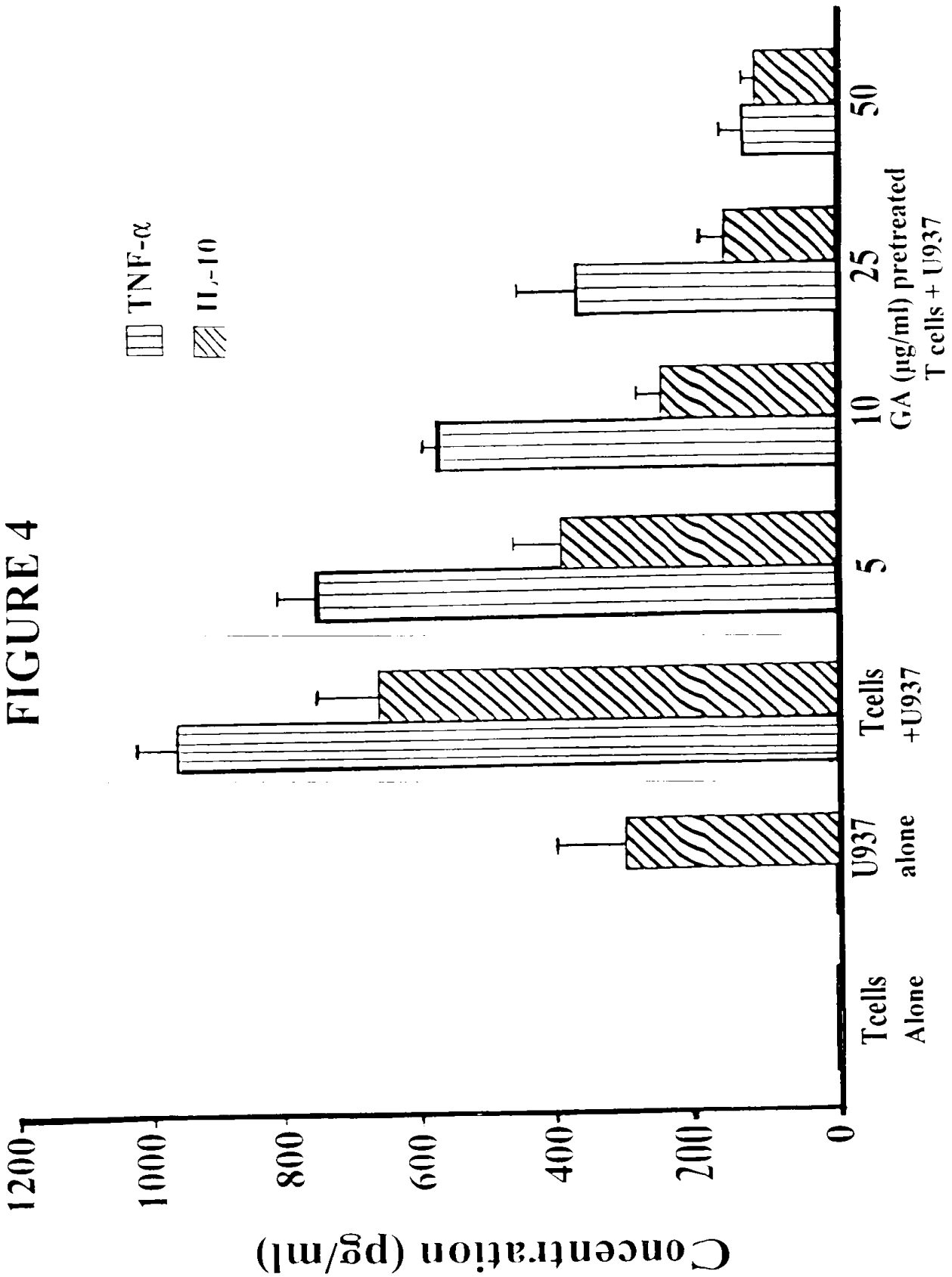

Concentration (pg/ml)
1200
1000
800
600
400
200
0
TNF-α
IL-10
Tcells Alone
U937 alone
Tcells +U937
5
10
25
50
GA (μg/ml) pretreated T cells + U937

FIGURE 5

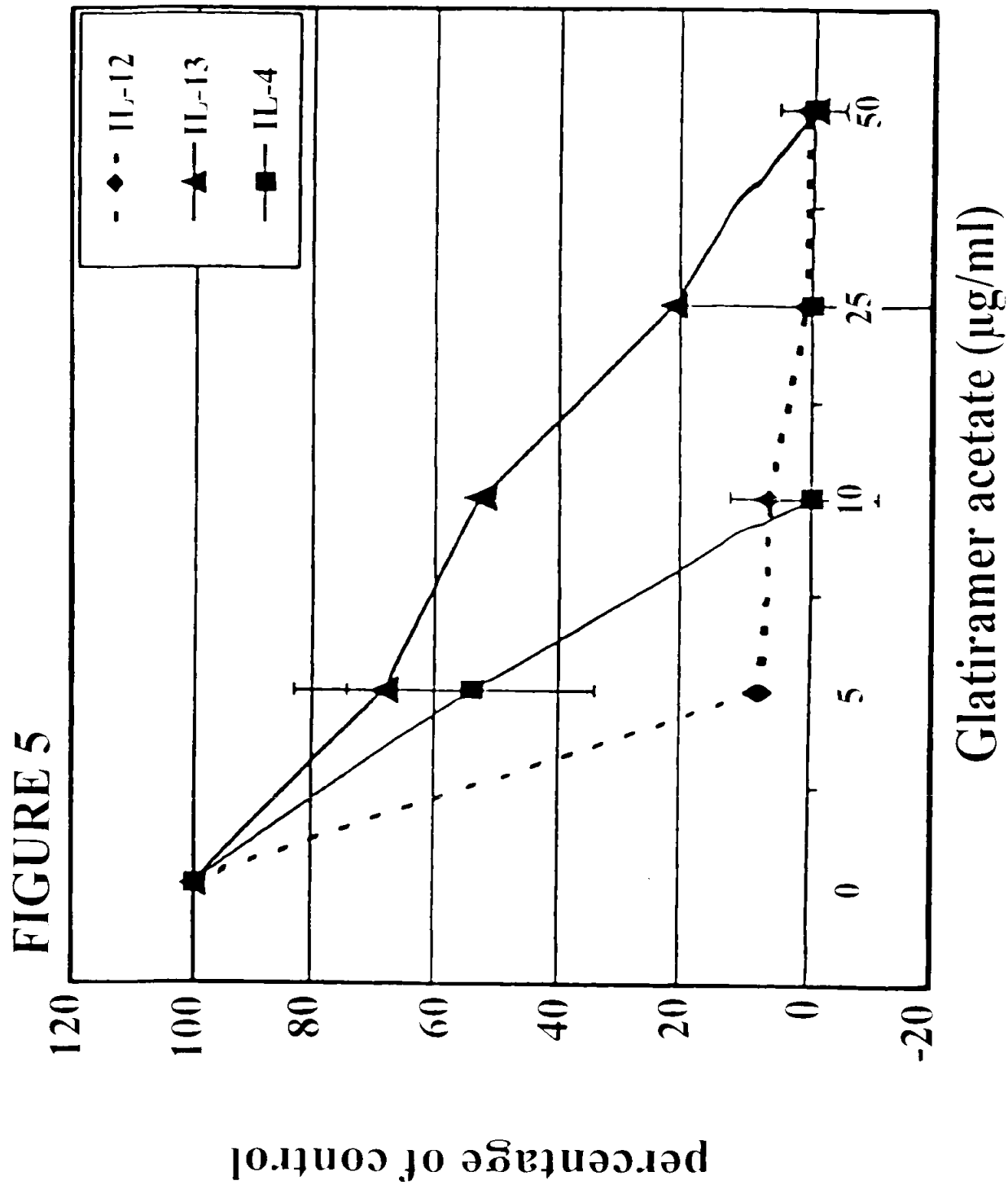

IL-12
IL-13
IL-4
120
100
80
60
40
20
0
-20
percentage of control
0
5
10
25
50
Glatiramer acetate (µg/ml)

FIGURE 6

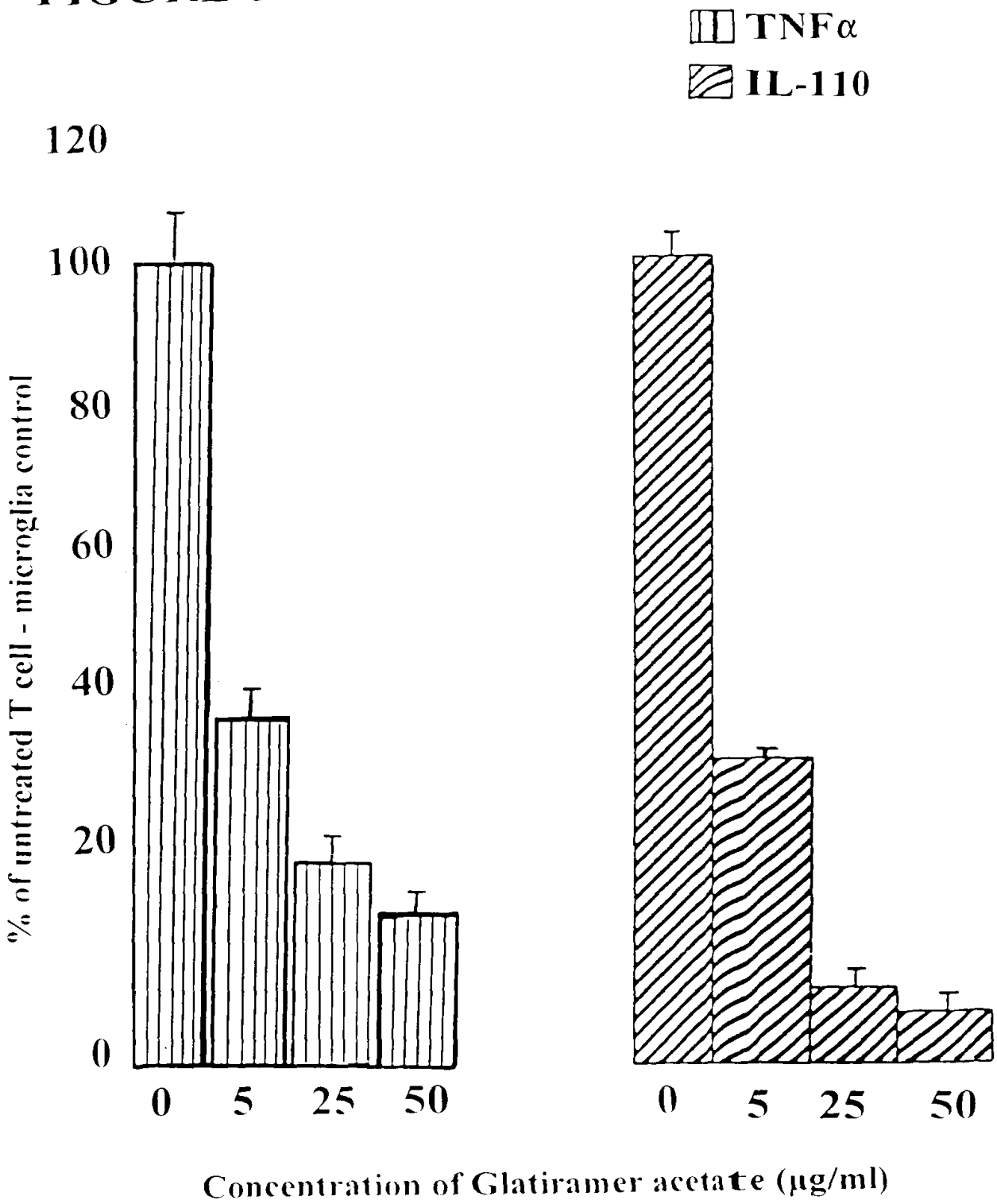

TNFα
IL-110
120
100
80
60
40
20
0
% of untreated T cell - microglia control
0
5
25
50
0
5
25
50
Concentration of Glatiramer acetate (µg/ml)

FIGURE 7

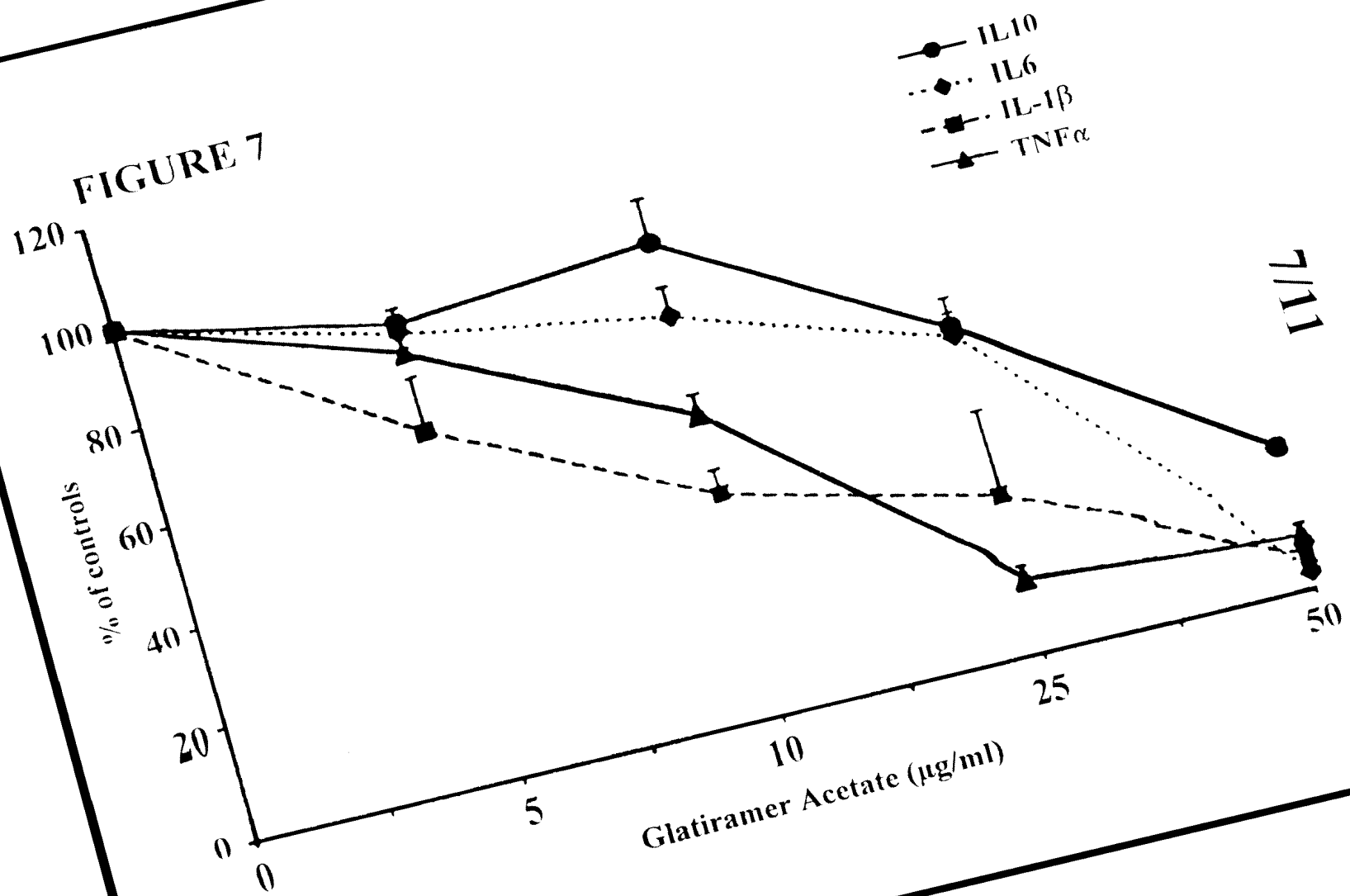

IL10
IL6
IL-1β
TNFα
120
100
80
60
40
20
0
% of controls
0
5
10
25
50
Glatiramer Acetate (µg/ml)

FIGURE 8A

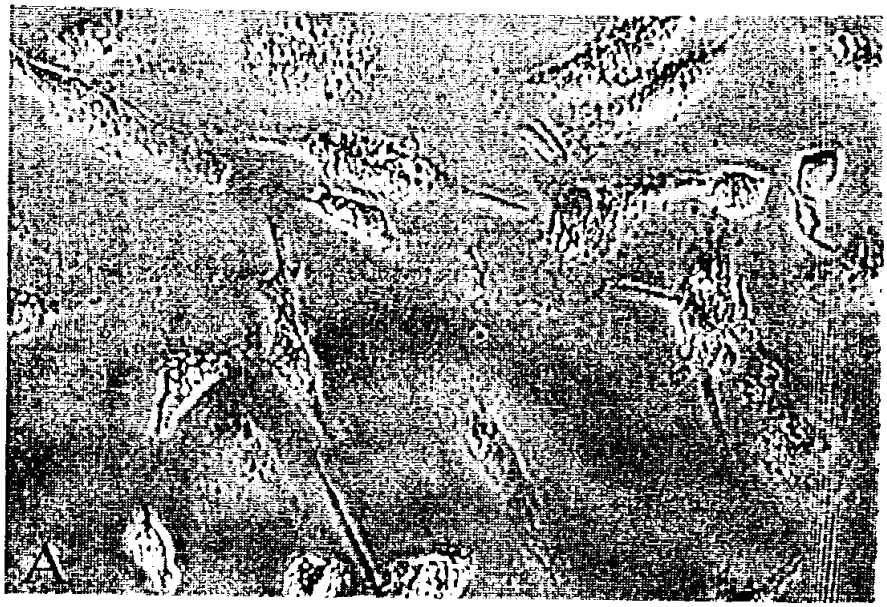


FIGURE 8B

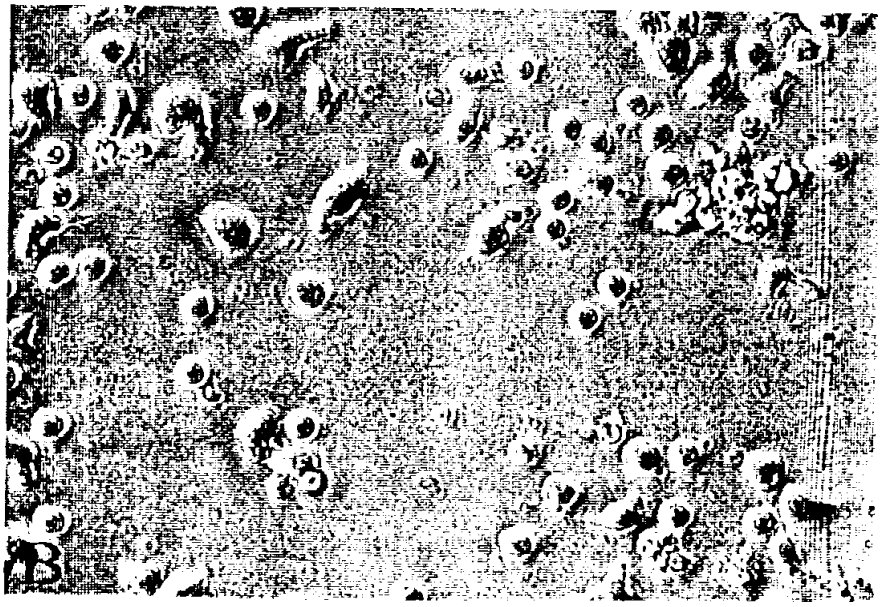


FIGURE 8C

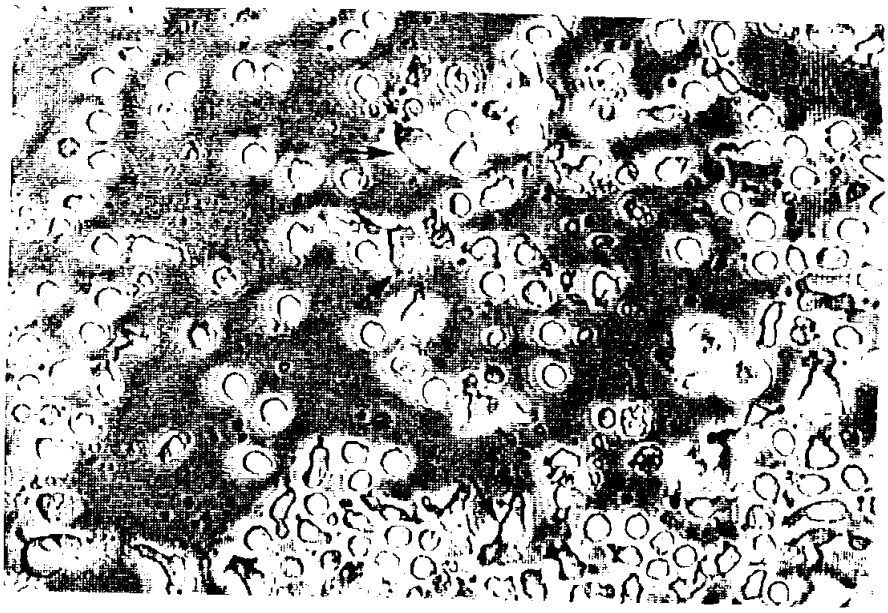


FIGURE 8D

FIGURE 9

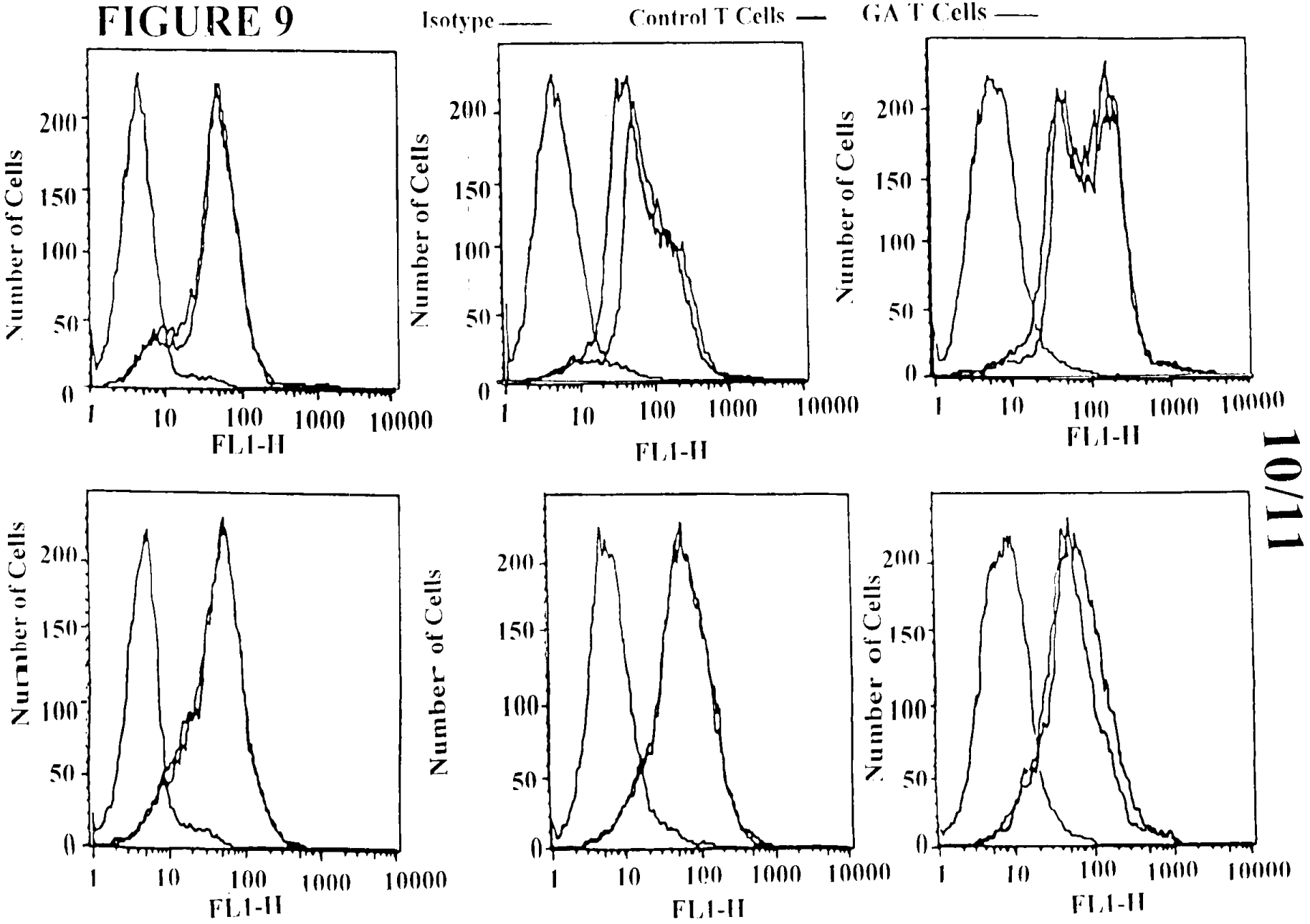
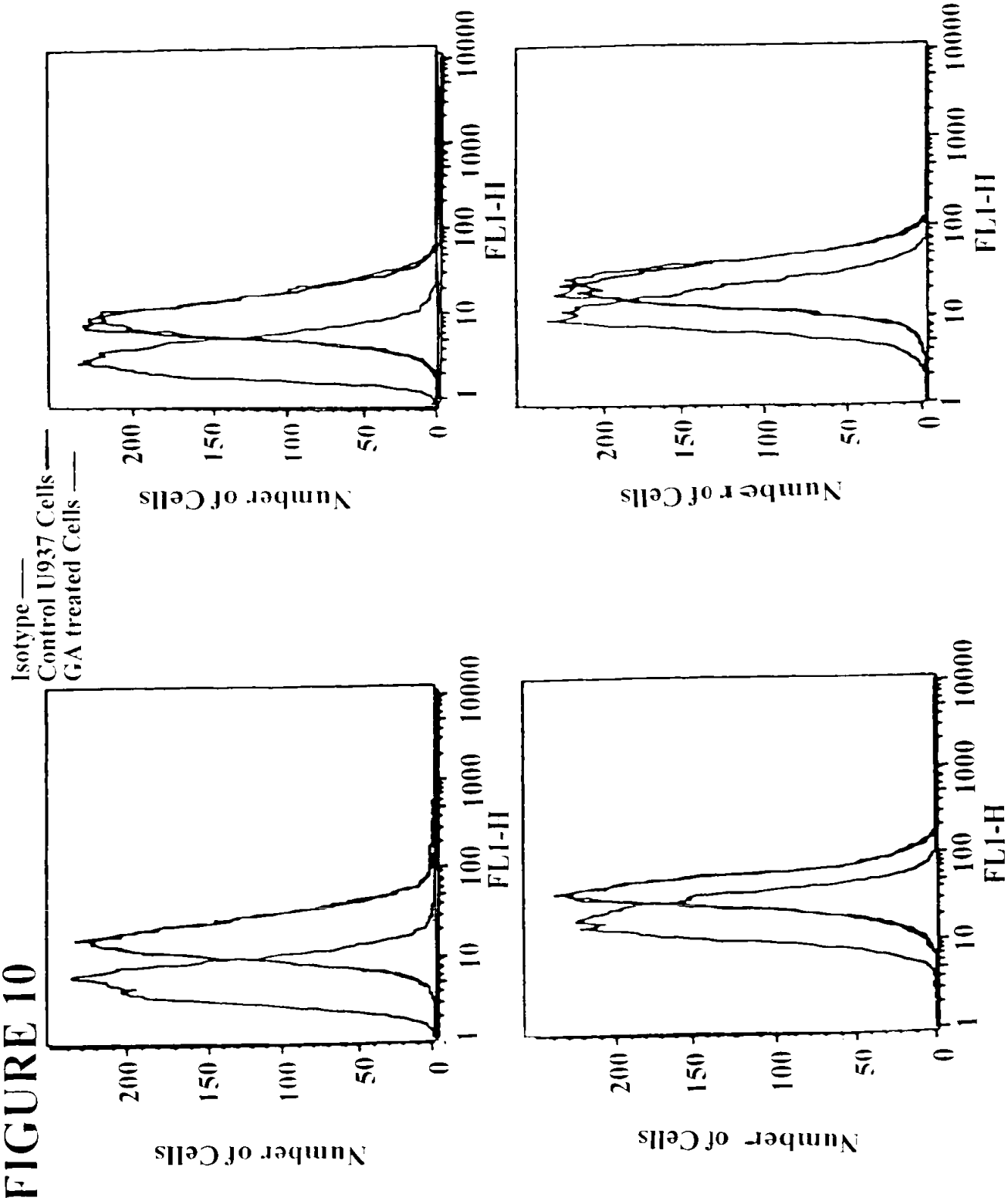

Isotype
Control T Cells
GA T Cells
Number of Cells
FL1-H
0
50
100
150
200
1
10
100
1000
10000

FIGURE 10


Isotype
Control U937 Cells
GA treated Cells
Number of Cells
FL1-H
0
50
100
150
200
1
10
100
1000
10000